

SOME POTENTIALLY SERIOUS DISEASE PROBLEMS IN THE CULTURE OF PENAEID SHRIMP IN NORTH AMERICA¹

DONALD V. LIGHTNER²

INTRODUCTION

Successful commercial culture of shrimp is not yet a reality in North America. The ever-increasing demand for shrimp coupled with rising prices for shrimp has lead to increased interest in the development of commercially viable shrimp farms (Neal, 1973a). Shrimp farms using the traditional pond culture approach on a pilot scale are located in Florida, Louisiana, and Texas. One promising approach to shrimp culture in North America is raceway culture (Neal, 1973b). This method is being studied in Galveston, Texas, and in Puerto Peñascol Sonora, Mexico.

Among the factors delaying successful development of shrimp culture in North America is the need for the development of methods for the diagnosis, treatment, and prevention of disease. At the present time there are at least five major disease of penaeid shrimp in North America that are likely to pose obstacles to successful commercial culture. These five disease are: (1) a mycosis of larval shrimp caused by a *Lagenidium* sp.; (2) mycotic infection of juvenile shrimp with *Fusarium* spp.; (3) bacterial infections caused by *Vibrio* spp. and *Beneckea* spp.; (4) a complex of several gill diseases the causes of which individually or collectively result in respiratory failure; and (5) the "cotton shrimp" group of diseases caused by several species of microsporidia.

MATERIALS AND METHODS

Most of the shrimp for these studies were obtained from the National Marine Fisheries Service shrimp hatchery and rearing facility at Galveston, Texas or from the University of Arizona-University of Sonora experimental shrimp farms at Tucson, Arizona and Puerto Peñasco, Sonora, Mexico. Additional shrimp were obtained from the Dow Chemical Company

experimental shrimp hatchery and rearing ponds at Freeport, Texas, and from the Texas Parks and Wildlife Department shrimp rearing ponds at Palacios, Texas. Some wild shrimp from commercial bait dealers on Galveston Bay were also used.

Methods of diagnosis and isolation, culture, and identification of presumed pathogens are given in the appropriate sections for each of the diseases discussed.

Shrimp selected for histological examination were fixed live in either 10% phosphate buffered formalin, Carnoy's fixative or Davidson's fixative. In the case of small shrimp (under 60mm in total length) the cuticle over the hepatopancreas and over the abdominal musculature and midgut was opened with scissors to enhance fixative penetration. In the case of larger shrimp, body regions that contained the organs or tissues of interest (the gills for example) were removed and fixed separately. Embedding, sectioning, and staining were accomplished using routine histological methods.

RESULTS AND DISCUSSION

Larval Mycosis

The occurrence of a *Lagenidium* sp. in penaeid shrimp was first observed in the spring of 1971 at the Dow Chemical Company's shrimp hatchery at Freeport, Texas (Cook, 1971). The fungus caused extensive mortality of brown shrimp (*Penaeus aztecus*) larvae within 2 to 3 days in hatchery tanks. The disease appeared sporadically during the remainder of 1971 and again in 1972 and 1973. Each time it appeared it resulted in high mortalities, sometimes nearly 100% (personal communication, Bruce Hysmith, present address: Texas Parks and Wildlife Department, Palacios, Texas).

In the summer of 1972 a fungus disease of larval white shrimp (*P. setiferus*) caused by a *Lagenidium* sp. occurred at the National Marine Fisheries Service shrimp hatchery in Galveston, Texas (Lightner and Fontaine, 1973). The presence of the disease first became apparent when the white shrimp larvae reached the second protozoal stage. The infection

¹ Contribution No. 395, National Marine Fisheries Service, Gulf Coastal Fisheries Center, Galveston Laboratory, Galveston, Texas 77550.

² National Marine Fisheries Service, Gulf Coastal Fisheries Center Galveston Laboratory, Galveston, Texas 77550

was limited to one hatchery tank and mortality due to the disease was 12%. Mortality stopped as the remaining larvae reached the first mysis stage. Despite the presence of large numbers of unencysted zoospores in the tank water at the time, none of the mysis stage larvae examined from several random samples were infected by the fungus.

Lagenidium disease of larval penaeids has also been reported from other shrimp hatcheries. Barkate, et al. (In Press) reported that the disease had occurred at the Ralston Purina hatchery at Crystal River, Florida. There the disease was responsible for complete mortality in one hatchery tank within 2 days after its appearance. On other occasions the disease

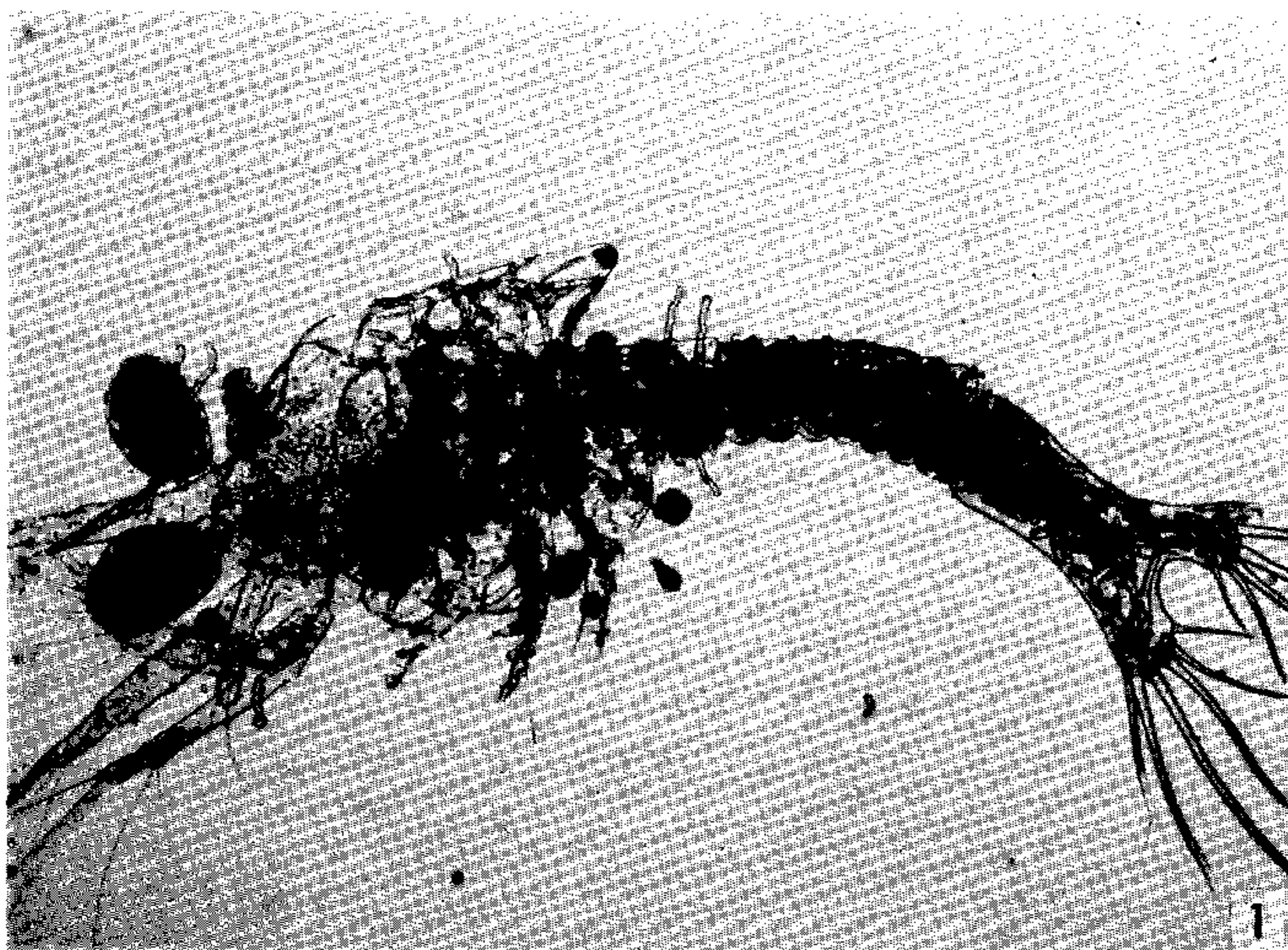


Figure 1. Larval white shrimp (protozoa II) heavily infected with a *Lagenidium* sp. Extramatrix hyphae, some with terminal vesicles, are shown protruding from the shrimp. No stain. $\times 72$.

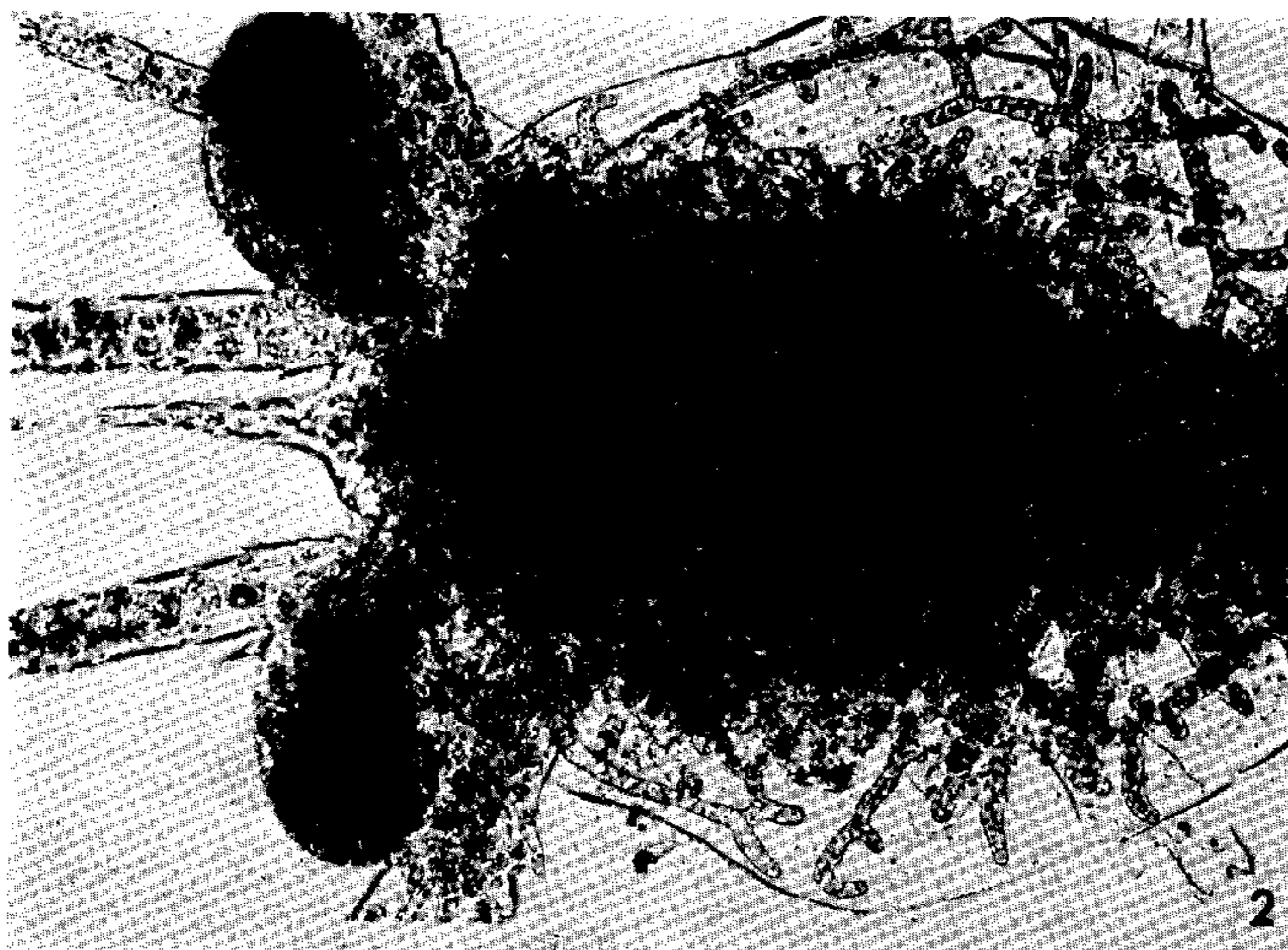


Figure 2. Larval white shrimp with hyphae of *Lagenidium* sp. occupying much of the space in the cephalothorax, eye stalks, and appendages. No stain. $\times 140$.

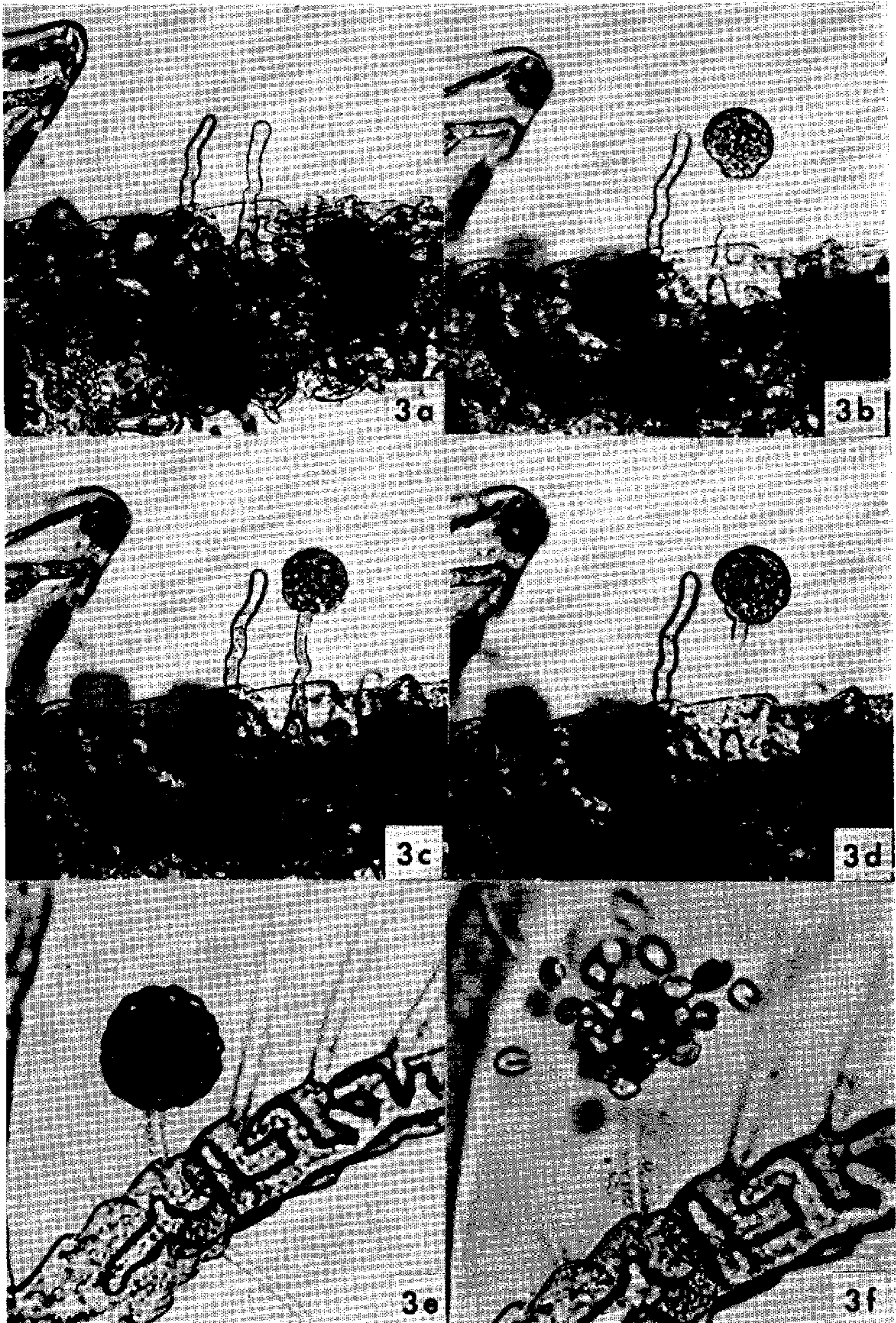


Figure 3. (a) An extramatrix "discharge tube" from the same shrimp as shown in Fig. 1. No Stain. $\times 220$; (b, c, d) A unit of cytoplasm is shown flowing through the "discharge tube" into the vesicle. No Stain. $\times 252$; (e) A vesicle in which the outline of individual planonts (zoospores) has become apparent. No Stain. $\times 315$; (f) The same vesicle as in 3c during planont discharge. The planonts are reniform and are motile by two flagella which arise from the lateral groove. No stain. $\times 800$.

was observed, but it was not always responsible for extensive mortalities. Barkate did not indicate what species of penaeids were involved in the epizootics at Crystal River.

Brown shrimp (*P. aztecus*) larvae were found to be highly susceptible to the fungus when exposed experimentally as protozoal stage I or stage II larvae. In one experiment in which 2,000 stage I protozoal larvae were exposed to zoospores and hyphae of the fungus, mortality reached 20% by 96 hours after inoculation. In a more recent experiment in which 4,000 stage I and stage II protozoal brown shrimp were exposed to zoospores of *Lagenidium*, a 97% mortality occurred by 96 hours after inoculation. Infection of mysis I larvae was observed in one latter experiment.

In natural and experimental epizootics in brown and white shrimp larvae, the protozoal stages seemed to be the most susceptible. Occasionally infected larvae in the last naupliar stage or the first mysis stage were observed, but infection of the protozoal stages was most typical.

The earliest sign of infection in a larval white or brown shrimp was the presence of hyphae within the appendages. Such individuals were much less active than noninfected controls. Infection occurred when a zoospore encysted upon a susceptible larva and germinated. If the germinal hyphae penetrated the cuticle, an infection was established. The fungal mycelium gradually invaded and replaced nearly all of the striated muscle tissue of the larval shrimp (Fig. 1). The thorax, abdomen, swimming appendages, and even the eye stalks became filled with hyphae (Figs. 1 and 2). Massive tissue destruction, particularly of the striated muscle, resulted in immobilization of the shrimp as much as 1 hour before death. An occasional movement of an appendage or contraction of the hindgut musculature were the only signs of life seen in these shrimp.

Soon after death of an infected larvae, the process of sporulation began with the emergence of "discharge tubes". The apical end of these "discharge tubes" swelled, forming a vesicle as it filled with individual units of cytoplasm which flowed from a sporangium located on an intramatrix hypha located within the body of the larva (Fig. 3). Planonts or zoospores developed from the amorphous mass of cytoplasm in the vesicle and were released when their movements ruptured the vesicle. Planonts were reniform, motile by two flagella that originated from the lateral groove, and were 8.7 by 12.0 μm in size (Fig. 3).

Lagenidium sp. from penaeids was easily isolated and cultured in several types of media. Isolation was facilitated by the addition of penicillin (500 units/ml

of medium) and streptomycin (500 $\mu\text{g/ml}$ of medium) to isolation medium to inhibit bacterial growth. For inoculation of isolation media, a single infected larva or a few milliliters of the seawater containing infected larvae and planonts was introduced into isolation medium. Best growth was obtained on Sabouraud dextrose agar enriched with 2% NaCl and shrimp homogenate. On this medium growth of the fungus was rapid and the mycelium typically had covered the entire agar surface of a 100mm plate by 4–5 days after inoculation and incubation at 28°C (Fig. 4).

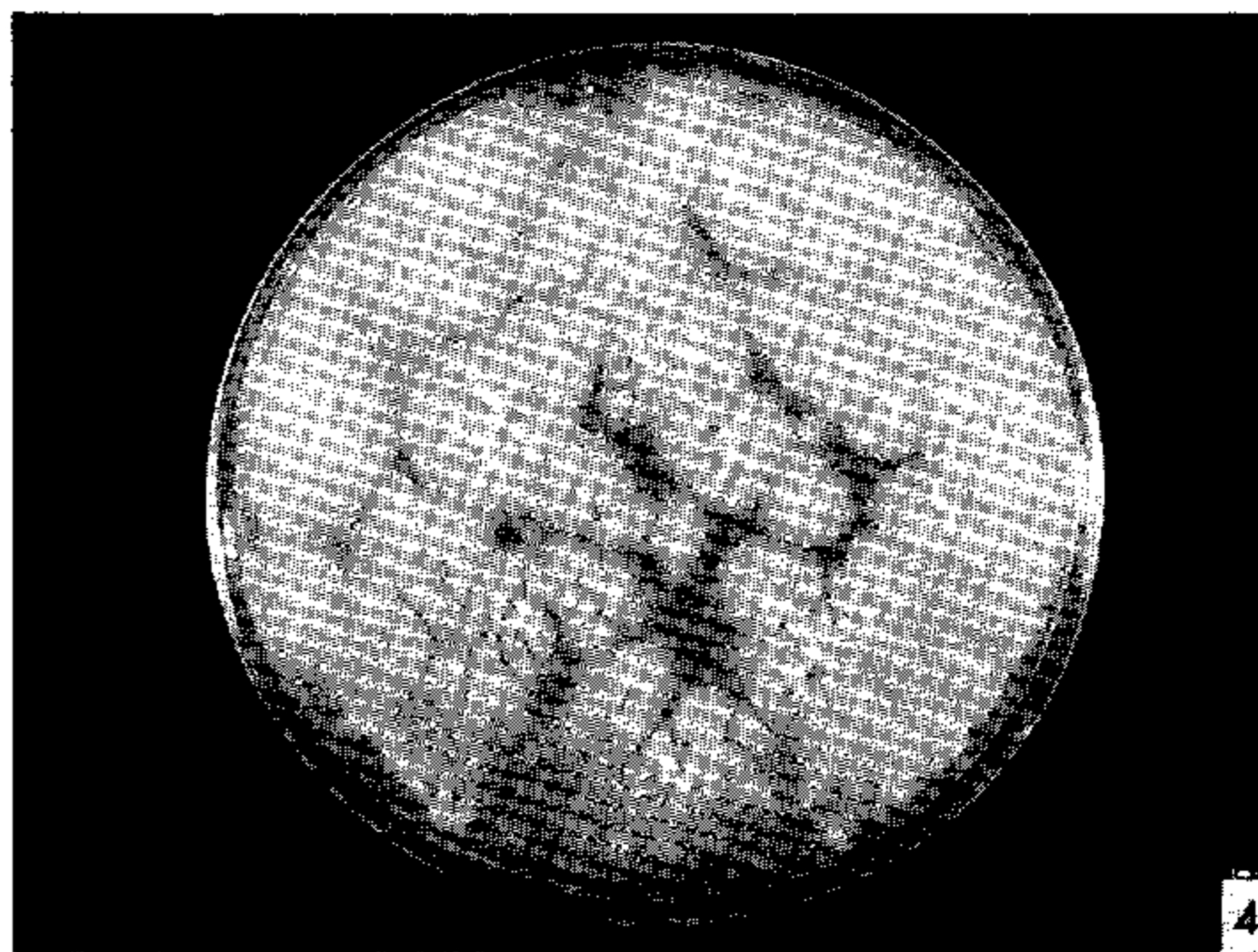


Figure 4. A 5-day-old culture of the *Lagenidium* sp. from the white shrimp (*P. setiferus*). Culture medium was Sabouraud dextrose agar enriched with 2% NaCl and shrimp homogenate. Incubation temperature was 28°C.

Means of control or treatment of *Lagenidium* infections in larval penaeids are not known and, as yet, the method by which the fungus is introduced into shrimp hatchery tanks has not been determined. Cultures made of ovary homogenates from spawning female brown shrimp have been negative for *Lagenidium* even when the larvae obtained from these females later developed the disease. A closely related species, *Lagenidium callinectes*, has been reported from the eggs and larvae of the blue crab, *Callinectes sapidus*, (Couch, 1942; Rogers-Talbert, 1948) and from the eggs of a barnacle (*Chelonibia patula*) that occurs commonly on the carapace of blue crabs and other marine animals (Johnson and Bonner, 1960). Another closely related species *L. chthamalophilum* has been reported as a parasite of the ova of the barnacle *Chthamalus fragilis* (Johnson, 1958). It is possible that various species of crabs and barnacles that occur naturally in the water source for shrimp hatcheries carry the *Lagenidium* sp. that is pathogenic to penaeid shrimp larvae as a normal parasite of their egg masses and hence serve as a reservoir for the parasite.

To date, all treatments of affected larvae tested

have been unsuccessful. All of the promising broad-spectrum antibiotics and fungicides tested have been determined to be toxic to penaeid shrimp larvae at the concentrations needed to kill or inhibit growth of the fungus. One chemical which shows promise in controlling the fungus is malachite green oxalate. Malachite green oxalate concentrations of 0.01 to 0.06 ppm (Bland, in press) was found to be effective in inhibiting the growth of *Lagenidium* sp. from white shrimp, but its toxicity to larval penaeids has not been tested.

***Fusarium* Disease**

Imperfect fungi belonging to the genus *Fusarium* have been reported from the Kuruma prawn, *Penaeus japonicus*, in Japan (Egusa and Ueda, 1972) and from laboratory-held pink shrimp, *P. duorarum*, in Texas (Johnson, 1974b). An additional *Fusarium* sp. has been described from the lobster, *Homarus americanus*, from a small experimental lobster farm in New York (Lightner, in press; Lightner and Fontaine, in press).

The *Fusarium* sp. in *P. japonicus* seemed to be a new species and the fungus was designated BG-Fusarium (Black Gill Fusarium) until it could be named (Egusa and Ueda, 1972). As the name "Black Gill Fusarium" implies, the fungus causes a disease that produces black gills in *P. japonicus*. The disease was shown to be the cause of serious mortalities among pond-cultured prawn populations. Affected parts of the gills carried septate hyphae of the fungus. Intramuscular inoculation of healthy prawns with conidia of the fungus caused "black gill disease", and the fungus was isolated from gill lesions of artificially infected prawns. On many culture media, including Sabouraud's dextrose agar medium, the fungus produced a dark purplish brown diffusible pigment.

The *Fusarium* sp. in pink shrimp (*P. duorarum*) did not cause a black gill condition but did infect the gills and the antennal scales. Less than 5% of the laboratory population were found to have the disease and in these the spread of the fungus was slow, taking up to 2 weeks to develop into more than 10% of the body area (Johnson, 1974b).

Lobsters (*H. americanus*), infected with a *Fusarium* sp. nearly identical morphologically to BG Fusarium (Lightner and Fontaine, in press), developed focal melanized cuticular lesions on the exoskeleton, appendages, and gills. A generalized "black gill" condition was not observed, although death of affected lobsters seemed to result from rapid antemortem growth of the fungus in the gills and consequent respiratory failure.

Cook (1971) reported an unidentified species of fungus that infected juvenile brown shrimp (*P. aztecus*) in the hatchery. Lesions due to the fungus

appeared as black spots and proved fatal when they spread to the gill region. This fungus may have been a *Fusarium* sp.

Another *Fusarium* sp. that differs morphologically from the *Fusarium* spp. of *P. japonicus*, *H. americanus*, or *P. duorarum* was shown to be the cause of a severe epizootic in the California brown shrimp (*P. californiensis*) in June and July of 1974, at the University of Arizona's experimental shrimp farm at Puerto Peñasco, Mexico. The disease was confined two raceways at the farm, but in one of the raceways nearly 100% incidence of infection was observed. Of the approximately 6,000 100mm shrimp present in this raceway when the disease first became apparent, only 600 survived to July 12. The fungus, a *Fusarium* sp., typically infected the gills, the coxal (basal) segments of the walking legs, and the body wall behind the gills and above the coxal segments. The coxal segment, gill process, and adjacent portions of the 14th segment (the segment having the last or 5th walking legs) were nearly always infected by the fungus (Fig. 5). Other areas, such as the gill cover and the ventrolateral portions of the first abdominal segment, as

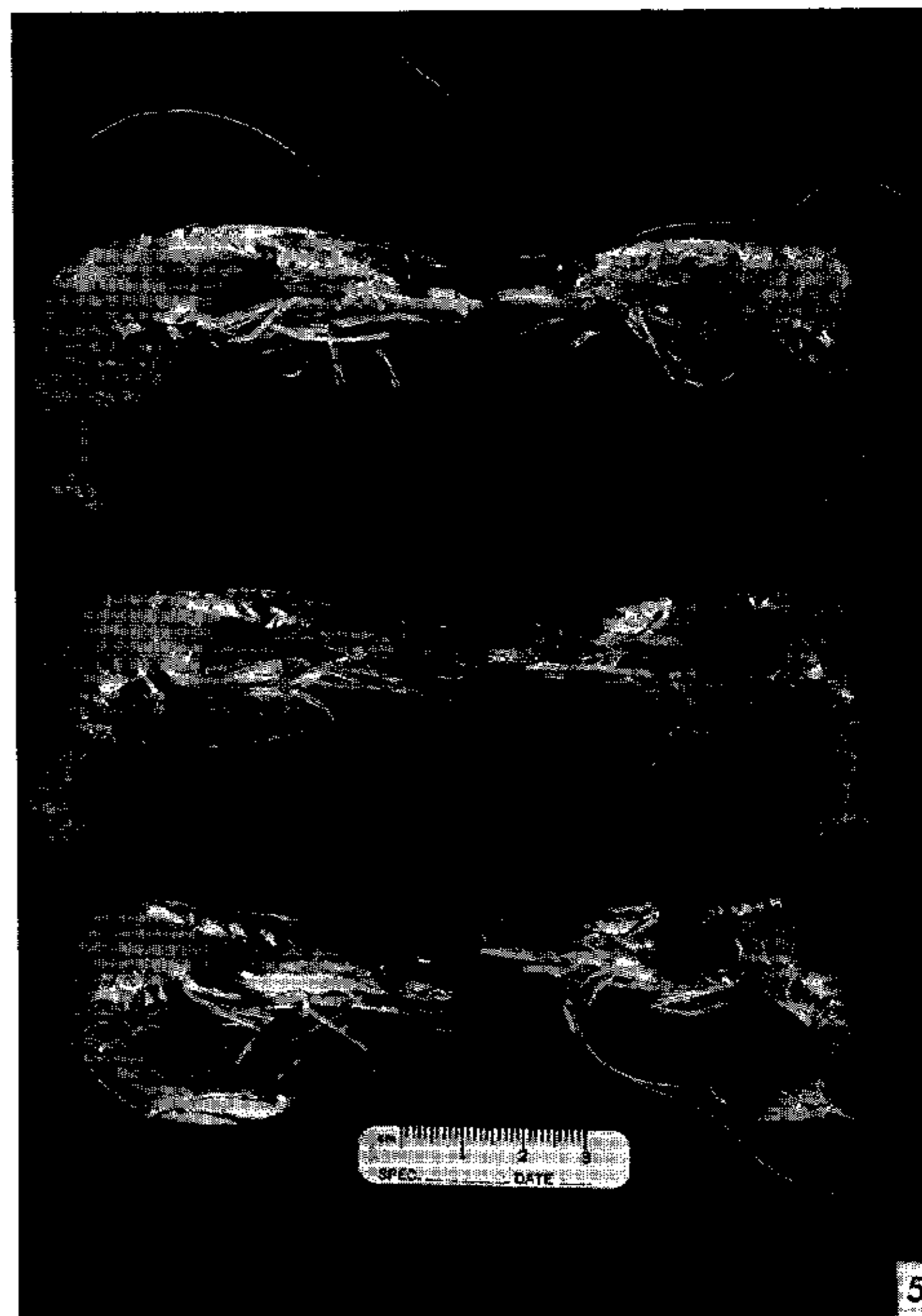


Figure 5. California brown shrimp (*P. californiensis*) infected with a *Fusarium* sp. Note the blackened gills, particularly above the 4th and 5th pereopods.

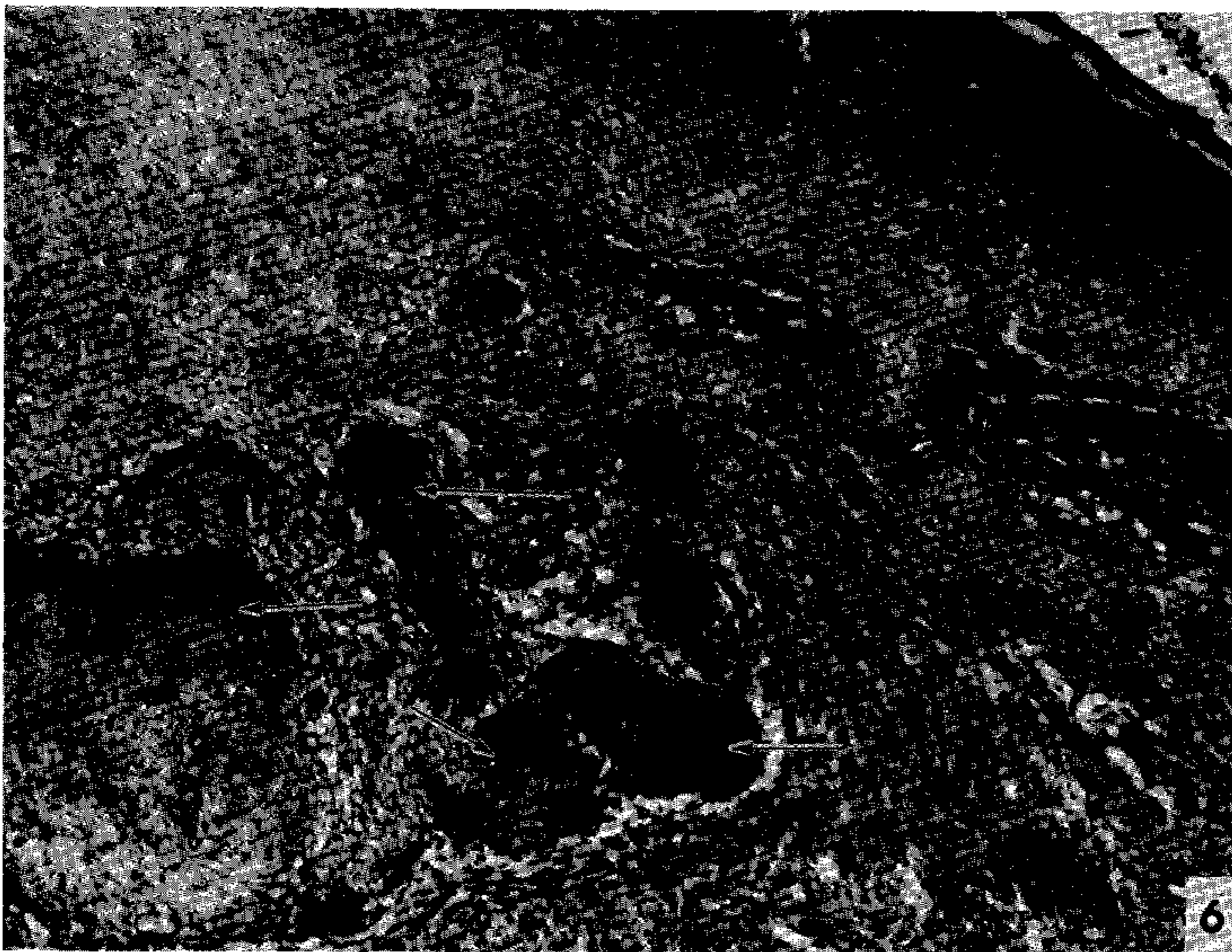


Figure 6. Photomicrograph of a histological section through a melanized *Fusarium* lesion in the body wall above the 5th pereopod and gill process. The dark band in the upper right corner is melanin. Masses of hemocytes are encapsulating hyphae (arrows) of the *Fusarium* sp. Some of these encapsulations are melanized near their centers. Hematoxylin and eosin. $\times 250$.



Figure 7. Photomicrograph of a wet mount of the gills of *P. californiensis* that died due to destruction of the gills by a *Fusarium* sp. Hyphae, microconidia, and macroconidia, and macroconidia are visible within the gill lamellae. No stain. $\times 840$.

well as wounds at various locations on the shrimp, were occasionally found to be infected by the fungus. In every instance, at least some of the lesions due to the fungus on a particular shrimp, were marked black by deposition of melanin. Hence, most of the affected shrimp showed at least a limited "black gill" condition (Fig. 5). In these black lesions (Fig. 6) the melanin deposition resulted from the activity of hemocytes responding to the presence of hyphae and to tissue destruction caused by the fungus. Encapsulation of hyphae was typical when hyphae were present in subcutaneous or muscle tissues. Death in affected shrimp, as with *Fusarium* infections in the Kuruma prawn and the lobster, probably resulted from destruction of the gills by a rapid antemortem growth of the fungus into the gill processes that was not accompanied by an appreciable hemocyte response (Fig. 7).

The fungus was isolated in pure culture from gill lesions of every shrimp sampled that had lesions like those described above. Isolation media were Sabouraud dextrose agar supplemented with 2% NaCl and shrimp homogenate (SSS medium) and Cantino PYG both supplemented with 2% NaCl. Penicillin and streptomycin were added to isolation media to inhibit bacterial growth. Large numbers of macroconidia were produced by this *Fusarium* sp. on SSS media. From a single 100mm diameter SSS plate after 10

days of incubation at 28°C, 5.8×10^9 macroconidia were recovered from saline washings of the agar surface.

The *Fusarium* sp. from *P. californiensis* produced micro and macroconidia in artificial media and in shrimp tissues. Microconidia were typically ovoid to oblong and frequently slightly curved (Fig. 8); they were one-celled, or two-celled, and ranged from 9 to 18 μ m in length. Macroconidia were typically three-celled or four-celled and canoe shaped, or occasionally, cigar shaped. Macroconidia ranged in length from 30 to 47 μ m (Fig. 9). The fungus produced a pale brown diffusible pigment on SSS medium. This pigment is much paler than the dark purplish brown pigment produced by the *Fusarium* sp. from *P. japonicus* (Egusa and Ueda, 1972)

The probable source of conidia that infected the shrimp was determined. At Puerto Peñasco sets of two raceways were enclosed in a single air-inflated plastic greenhouse. Water flowed through the raceways and drained into a common sump at the end of the greenhouse. The blower that inflated the greenhouse was located in the outside wall of the sump. Hence, air currents carried spray from the sump over the raceways where much of the spray settled. The *Fusarium* sp. was cultured from water and debris in the sump, from spray in the sump, and from the air above the raceways. SSS plates exposed to the air



Figure 8. Photomicrograph of microconidia of the *Fusarium* sp. from *P. californiensis*. From PYG broth culture. No stain. $\times 300$.

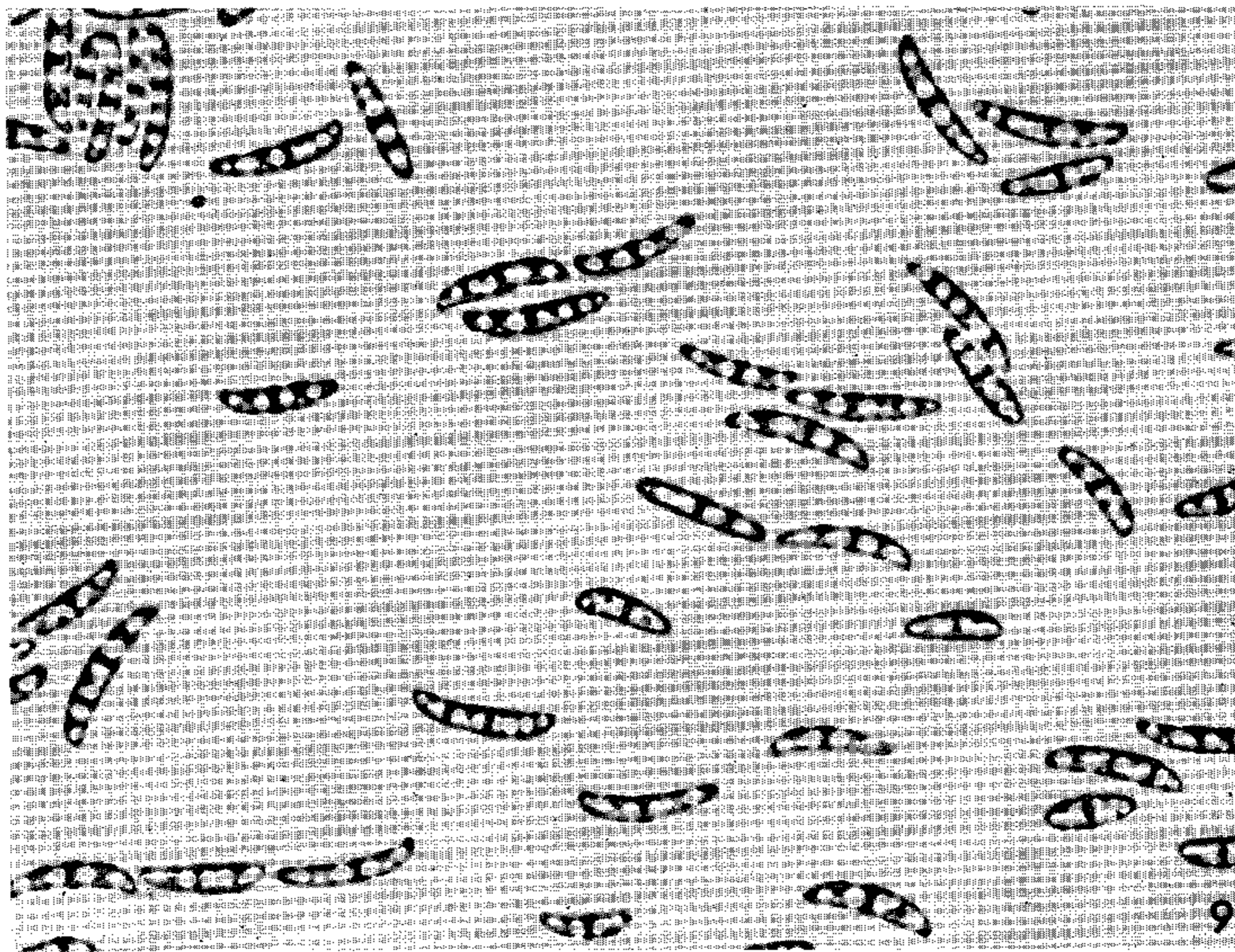


Figure 9. Photomicrograph of macroconidia of the *Fusarium* sp. from *P. californiensis*. From Sabouraud dextrose agar enriched with 2% NaCl and shrimp homogenate. No stain. $\times 500$.

in the greenhouse for only 10 minutes had colonies of the *Fusarium* sp. after 48 hours incubation at 28°C.

No effective treatments for shrimp infected with *Fusarium* have been developed. Malachite green oxalate at concentrations of 0.05 to 0.1 ppm for 24 hours appeared to be effective against exposed spores and hyphae that were present in the water and tanks, but internal hyphae and spores were not affected by this treatment. Control of the disease in the raceway system described was accomplished by elimination of sources of spores of the fungus and by destruction of shrimp infected with the fungus.

Bacterial Infections

Vibrio infections have been implicated as a major cause of mortality in juvenile penaeids in shrimp culture (Sindermann, 1971; 1974). *Vibrio parahaemolyticus*, the cause of an infectious food poisoning syndrome in Japan (Nickelson and Vanderzant, 1971), was isolated from white shrimp (*P. setiferus*) taken from Galveston Bay, Texas (Vanderzant, et al., 1970a). The same organism was pathogenic to brown shrimp (*P. aztecus*) when bits of frozen white shrimp infected with the organism were fed to brown shrimp, or when cultures of the organism were added to aquaria with brown shrimp.

Lewis (1973a) reported experiments in which adult brown shrimp were challenged with a field isolate of *Vibrio anguillarum*. One-tenth milliliter of a 24-hour

broth culture diluted 100-fold and introduced by injection beneath the dorsal carapace at the terminus of the rostral groove caused death of the shrimp within 5 days.

The normal microbial flora of brown and white shrimp from the Gulf of Mexico and from pond-reared brown shrimp has been studied (Vanderzant, et al., 1970b; Vanderzant et al., 1971). In these studies *Vibrio* spp. were among the predominant isolates from pond-reared brown shrimp, but were apparently not a significant part of the normal flora of brown and white shrimp from the Gulf of Mexico.

Vibrio alginolyticus has been implicated as the cause of several large mortalities during 1972 and 1973 in hatchery-reared brown and white shrimp at the Dow Chemical Company shrimp hatchery in Freeport and at the National Marine Fisheries Service laboratory in Galveston, Texas. In the most severe epizootic, a 99% loss occurred over a 2-week period in a group of 100,000, 26mm (total length) brown shrimp (Lightner and Lewis, in press). *V. alginolyticus* was also frequently isolated from dead or dying wild brown, white and pink shrimp obtained from Galveston area commercial bait dealers. Other opportunistic bacterial species such as *V. alginolyticus*, *V. anguillarum*, *Aeromonas* sp., and *Pseudomonas* spp. have occasionally been isolated from dead or dying hatchery-reared or wild shrimp, but *V. alginolyticus* was

Table 1. Organisms isolated and source of penaeid shrimp exhibiting clinical signs of a bacterial septicemia.

Organism	Species of shrimp ¹ and total length	Source, number of shrimp affected, ² and percent mortality
<i>Vibrio alginolyticus</i>	B (40mm)	Lab-reared (NMFS); 150; 20%
<i>V. alginolyticus</i> , <i>V. anguillarum</i>	W (100mm)	Live bait dealer (Galv.); 50; 50%
<i>V. alginolyticus</i>	B (26mm)	Lab-reared (Dow); 100,000; 99%
<i>V. alginolyticus</i>	B (58mm)	Lab-reared (NMFS); 200; 40%
<i>V. anguillarum</i> , <i>Aeromonas</i> sp.	B (90mm)	Lab-reared (NMFS); 10; 10%
<i>V. alginolyticus</i>	W, P, B (100mm)	Live bait dealer (Galv.); 60; 50%
<i>V. alginolyticus</i>	B (41mm)	Lab-reared (NMFS); 1,500; 99%
<i>Vibrio</i> sp.	W, P (120mm)	Live bait dealer (Galv.); 100; 10%
<i>V. alginolyticus</i> , <i>Beneckeia</i> sp.*	B (10mm)	Lab-reared (Dow); 1,035,000; 64%
<i>V. alginolyticus</i>	W (100mm)	Live bait dealer (Galv.); 14; 100%
<i>V. alginolyticus</i> , <i>Pseudomonas</i> sp.	B (5mm)	Lab-reared (NMFS); 500,000; 35%
<i>V. anguillarum</i> , <i>A. formicans</i>	B (80mm)	Lab-reared (NMFS); 3; 100%

¹ B=brown shrimp (*Penaeus aztecus*), W=white shrimp (*P. setiferus*), and P=pink shrimp (*P. duorarum*). Average total length in parentheses.

² NMFS=National Marine Fisheries Service, Galveston, Texas, Dow=Dow Chemical Co. experimental shrimp hatchery and rearing unit, Freeport, Texas, and Galv.=Galveston, Texas.

* Possesses chitinase activity.

the most prevalent organism isolated from shrimp that showed clinical signs of a bacteremia (Table 1).

All the bacteria isolated from the hemolymph of moribund shrimp were Kovac's oxidase positive, were motile by polar flagella, and initially required the presence of at least 2% NaCl in the medium for growth. None of the fermentative bacteria produced gas in glucose, lactose, sucrose, or mannitol. Those organisms which failed to produce lysine decarboxylase were beta hemolytic on 5% bovine blood agar and on the basis of their ability to produce arginine dihydrolase, 2-3 butanediol, gelatinase, and indole were identified as *Aeromonas* sp. (Eddy, 1969; Eddy and Carpenter, 1964; Schubert, 1967). Those organisms identified as *Vibrio* sp. were sensitive to 2, 4-diamino-6, 7-diisopropyl pteridine phosphate (Schubert, 1962); produced lysine, ornithine decarboxylase, and indole; fermented sucrose; and grew in trypticase soy broth containing 10% NaCl. Further identification of the isolates was accomplished using methods described by Lewis (1973b).

The first apparent clinical sign of a lethal bacteremia was a gradual change from the usual colorless translucent appearance of the musculature, to a whitish-opaque coloration. Some infected animals examined also showed melanized cuticular erosions, and melanization of gill filaments and ventrolateral edges of the carapace. A slight darkening of the dorsal portions of the integument (due to expansion of integumental melanophores) and a reddening of the pereopods and the pleopods (due to expansion of integumental erythrophores) was apparent in moribund or freshly

dead shrimp with a bacteremia. Moribund shrimp commonly exhibited a pronounced dorsal flexure of the abdomen with the second and third abdominal segments at the apex of the flexure.

Behavioral signs of stress associated with the disease became more apparent as the disease progressed. These signs included reduced swimming activity, disorientation while swimming, and swimming on one side. Eventually, affected shrimp came to rest motionless on the bottom, some in an upright position supported by the pereopods, pleopods, and uropods, while others lay on their side. Some of these shrimp could be induced to brief periods of swimming activity by prodding. Death usually occurred 2 to 4 hours after the shrimp had become lethargic. Occasionally shrimp remained in the upright position even after death.

Hemolymph drawn with a tuberculine syringe directly from the heart of moribund shrimp having a bacteremia was slightly turbid in appearance and lacked the blue coloration that appears in clotted hemolymph of healthy shrimp. Typically, the hemolymph from moribund shrimp having a bacteremia required more time to clot than the hemolymph from healthy shrimp, and often it did not clot at all. Giemsa-stained hemolymph smears from moribund bacteremic shrimp contained hemocytes although in greatly reduced numbers compared to normal shrimp. Gram-stained hemolymph smears from the same animals contained numerous Gram-negative rods. Pure cultures of bacteria could usually be obtained from hemolymph drawn directly from the heart of

bacteremic moribund shrimp. Cultures made from impression smears of small pieces of muscle tissue aseptically removed from the abdomen of small shrimp (under 40mm total length) also frequently provided pure cultures of the presumed causative agent. Isolation medium was tryptic soy agar with 2% NaCl.

Addition of bacterial isolates to aquarium water or feeding of bacterial isolates to experimental shrimp seldom resulted in clinical disease. Clinical disease could only be produced by direct injection of about 10^4 bacterial cells into the abdominal muscle or hemocoel of an experimental shrimp. Other investigators have reported similar difficulties in infectivity experiments with decapod crustaceans. Barkate (1972) was unable to infect juvenile pink shrimp with *V. parahaemolyticus* when added to tank water at 10^4 cells per milliliter. Lewis (1973a) for the same reason selected injection of *Vibrio anguillarum* into experimental shrimp over other methods of exposure. Sniesko and Taylor (1947) were unable to infect American lobsters with *Pediococcus* (*Gaffkya*) *homari* introduced with the food, but succeeded in transmitting geffkemia disease to healthy lobsters by injection of bacteria. Later it was learned that the gaffkemia organism is transmitted only through ruptures in the integument and not through the consumption of infected food (Stewart and Rabin, 1970).

Our experience has shown that handling of otherwise healthy hatchery-reared shrimp occasionally results in the onset of a bacteremia due in most cases to a *Vibrio* sp. In all cases some sort of physical or chemical stress or injury preceded the onset of clinical disease. The capture and holding in tanks of wild penaeid shrimp often result in the same disease syndrome. Slight injuries resulting in interruption of the cuticle certainly occur when shrimp are subjected to rough handling or crowding in tanks. Cuticular injuries may provide a route of entry for potentially pathogenic bacteria which are a normal part of the microbial flora of pond-reared or hatchery-reared shrimp (Vanderzant, et al., 1970b; Vanderzant, et al. 1971).

Treatment of *Vibrio* infections is possible the addition of antibiotics to the ration or directly to the water. Experimental groups of brown shrimp fed Terramycin¹ at the rate of 360–387 mg/kg body weight/day for 14 days suffered less mortality than comparable control groups not fed antibiotic when challenged by direct intramuscular inoculation of at least 10^4 cells of *Vibrio alginolyticus* per shrimp

(Corliss, et al., in press). Chan and Lawrence (in press) reported the effectiveness of oxytetracycline-oleandomycin combinations in reducing bacterial populations in larval shrimp cultures and suggested that the antibiotic combination could be used to treat *Vibrio* and other bacterial infections in mysis and postlarval stage shrimp. Delves-Broughton (1974) reported that the broad spectrum antibiotic Furanace when added directly to the water is non-toxic and is rapidly absorbed into the tissue to treatment levels in *Macrobrachium rosenbergii*. The *Vibrio* spp. tested were all inhibited "in vitro" by less than 1 mg Furanace/liter. *Aeromonas* and *Cytophaga* spp. were inhibited by slightly higher concentrations of the chemotherapeutic (0.8 to 3.1 mg/liter). *Beneckea* spp. showed a varied response (3.1 to 12.5 mg/liter) while *Pediococcus* (*Gaffkya*) *homari* were resistant.

Shell Disease

Chitinoclastic bacteria are apparently a normal part of the microbial flora of the penaeids (Hood and Meyers, in press). However, several species of *Beneckea*, *Vibrio*, and *Pseudomonas* that produce chitinase have been isolated from shrimp exhibiting "shell disease" (Cook and Lofton, 1973). "Shell disease" was described by Rosen (1970) as a complex of closely related low virulence necrotic diseases of the integument of aquatic crustaceans. Rosen (1970) noted that chitinoclastic bacteria and fungi have been implicated as causative agents of the disease.

In 1973 an epizootic of shell disease developed at the experimental shrimp farm at Puerto Peñasco, Mexico. The epizootic appeared to be caused by a chitinoclastic variety of *Vibrio anguillarum*. In contrast to the forms of shell disease described by Cook and Lofton (1973) and Rosen (1970), the Peñasco variety of shell disease was accompanied by low but persistent daily mortalities of 1 to 5%. The disease appeared to be infections and not the result of secondary infections of wounds.

The lesions seen in the Puerto Peñasco variety of shell disease occurred consistently in certain locations, namely on the dorsal surface of the pleura of the first, second, and third abdominal segments, on the posterior edge of the lateral portions of the abdominal pleural plates, and along the dorsal surface of the branchial cavity (Figs. 10 and 11).

Shell disease at Puerto Peñasco has been treated experimentally with 1-hour static treatments of Hyamine 3500 at 5 ppm, potassium permanganate at 10 ppm, and mixtures of malachite green oxalate and formalin at 0.05 to 0.1 ppm and 20 to 75 ppm, respectively. Mixtures of malachite green oxalate and formalin at these levels have been effective in preliminary experiments in reducing losses due to shell

¹ Use of trade names in this publication does not imply endorsement of commercial products.

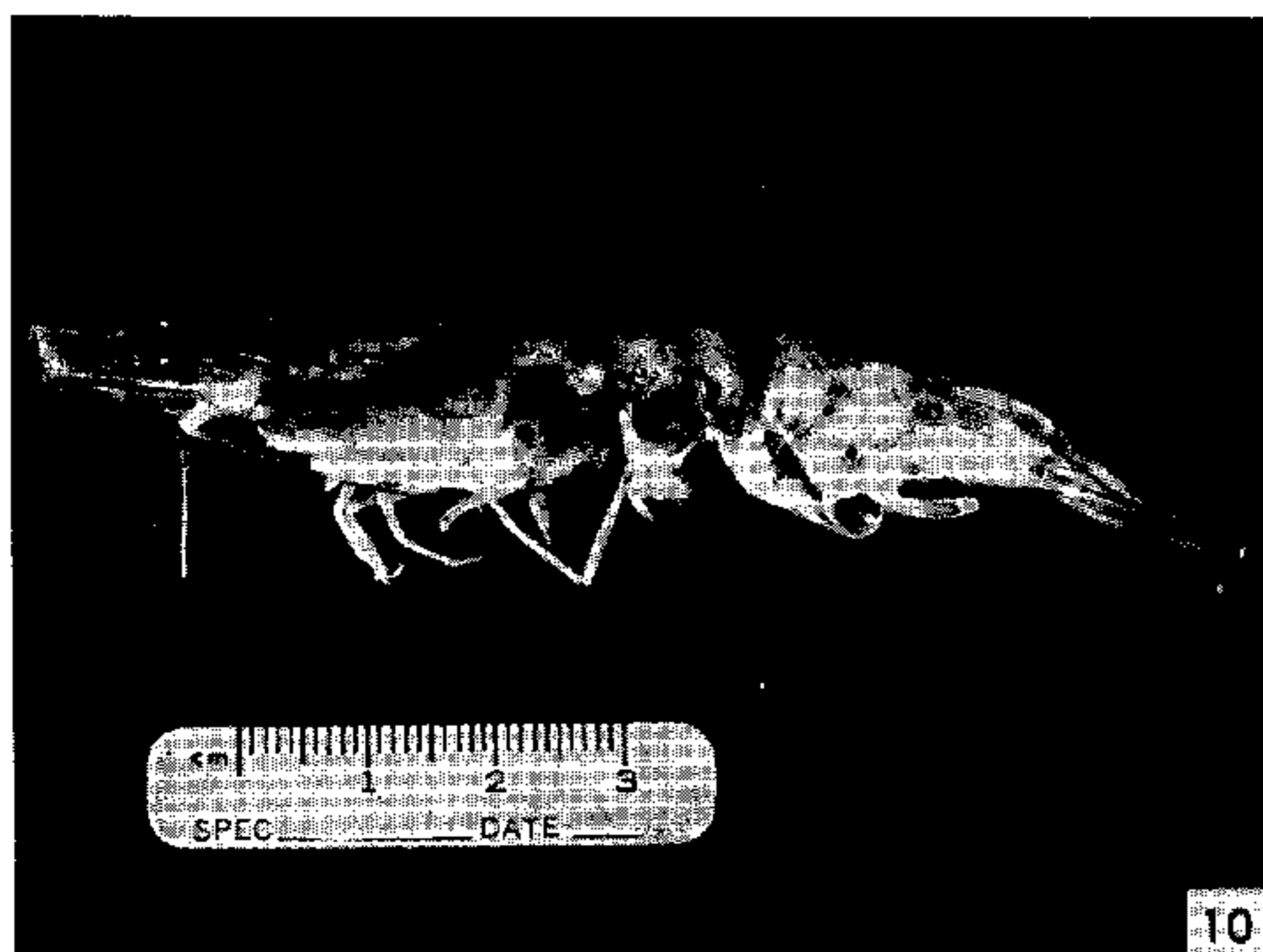


Figure 10. California brown shrimp (*P. californiensis*) with the Peñasco variety of shell disease. Melanized cuticular lesions typical of this form of shell disease are located on the plates of the abdomen, and in the dorsal portion of the branchial chamber.

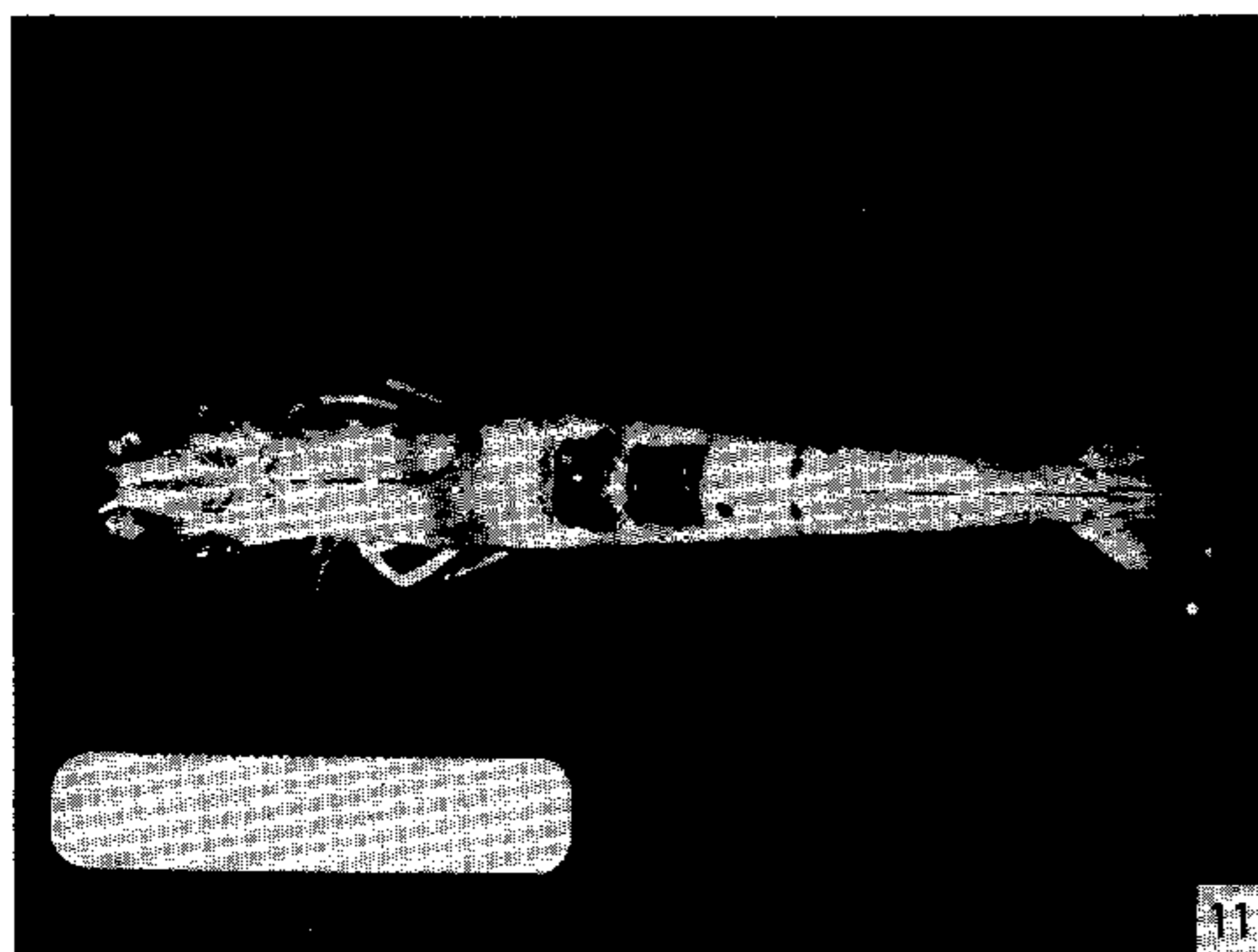


Figure 11. Dorsal view of a California brown shrimp with shell disease. The lesions shown on the dorsal portions of the first row abdominal segments were common in Peñasco shell disease epizootics.

disease. Following this treatment, shrimp having the disease molted within a few days and the new cuticle was free of lesions. Hyamine 3500 and potassium permanganate were not effective against shell disease at the concentrations tested.

Terramycin when added directly to the ration (20g Terramycin per 45kg ration; ration fed at approximately 10% of the biomass per day for 14 days) was also effective in a single preliminary experiment in treating shrimp having shell disease. Further studies using mixtures of malachite green oxalate-formalin and Terramycin (separately and together) as treatments for this form of shell disease are needed.

Gill Disease

Gill disease in penaeid shrimp is a complex of several diseases, any of which may result in death of

affected shrimp by destruction of the gills or by suffocation resulting from mechanical blockage of gas exchange across the surface of the gill lamellae. Organisms demonstrated to cause gill disease in penaeids include species of imperfect fungi belonging to the genus *Fusarium*, at least two types of ectocommensal peritrichs that belong to the genera *Zoothamnium* and *Lagenophrys*, and a filamentous bacterium that superficially resembles *Laucothrix mucor*.

"Black gills" or "black gill disease" has been described from several decapod crustaceans besides the penaeids (Johnson, 1974a; Egusa and Ueda, 1972; Uzman and Haynes, 1968). "Black gills" or melanization of the gill processes is a clinical sign of some types of gill disease, but is not a disease in itself. "Black gills" occur in shrimp having fungus infections of the gills (Egusa and Ueda, 1972; Uzman and Haynes, 1968), and in shrimp with heavy infestations of *Lagenophrys*.

"Black gills" are not usually seen in animals that have heavy infestations of the ectocommensal peritrich *Zoothamnium* or of the filamentous bacterium on the surface of the gills. Detritus and algae are often trapped by these ectocommensals resulting in gills that range in color from green to dark brown, but usually the gills are a very pale brown or colorless.

Gill Disease Due to Fusarium sp.—Gill disease due to species of *Fusarium* has been discussed earlier and is often accompanied by black gills.

Gill Disease Due to Epicomensal Protozoans.—Johnson et al. (1973) reported the loss of an estimated 2,000 pond-held brown and white shrimp in a single day due to the presence of large numbers of *Zoothamnium* sp. (Fig. 12) on the gills and to a reduction in dissolved oxygen. Mortality was attributed to anoxia as the mortalities occurred when the infestation of the protozoan became heavy enough to restrict oxygen exchange, and when the dissolved oxygen level in the ponds dropped below 3 ppm to a low of 2.6 ppm. In ponds where no *Zoothamnium* sp. were observed on the shrimp, no mortalities occurred despite the low dissolved oxygen levels. A dissolved oxygen level of 2.6 ppm is not normally lethal. Good survival has been experienced with *P. aztecus* in culture ponds even when the dissolved oxygen fell to 1 ppm.

Histopathological lesions of the gills, appendages, or of the general body surface have not been demonstrated at the site of attachment of a colony of *Zoothamnium* sp.. The stalks of colonies of this protozoan attach to the surface of the cuticle and do no mechanical damage. There is no foreign body response at the site of attachment by the shrimp's hemocytes (Fig. 13). Death occurs when the effective respiratory surface of the gills is reduced by the presence of

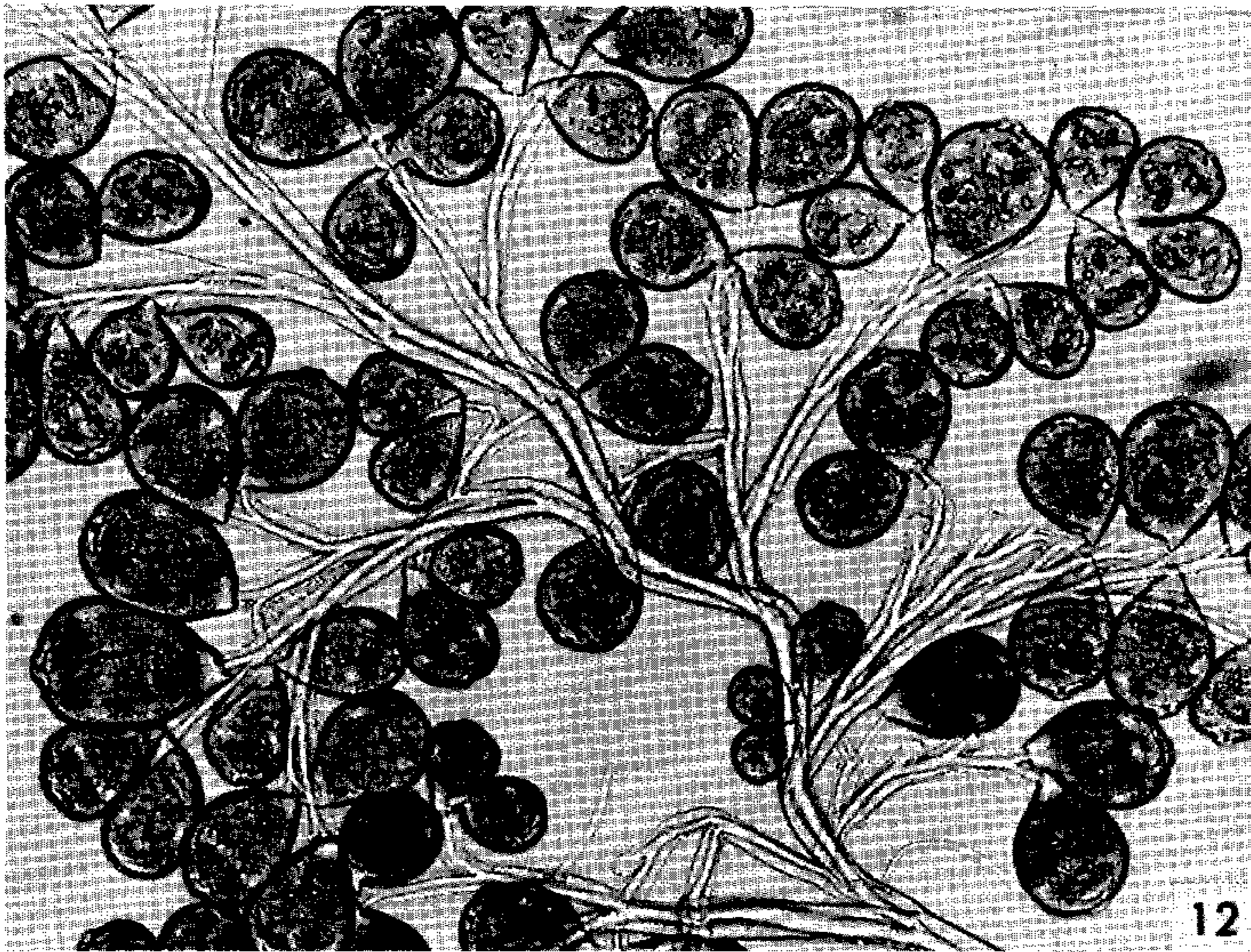


Figure 12. Photomicrograph of a wet mount preparation of *Zoothamnium* sp. from the gills of a brown shrimp (*P. aztecus*). No stain. $\times 320$.



Figure 13. Histological section of the gills of a white shrimp (*P. setiferus*). Colonies of *Zoothamnium* sp. are attached to the cuticle of the gill lamellae but host response is absent. Hematoxylin and eosin. $\times 250$.

numerous colonies of *Zoothamnium* sp. and suffocation results. The process is passive and is probably aggravated by reduced dissolved oxygen concentrations in the water (Overstreet, 1973).

Successful control of *Zoothamnium* sp. on penaeid shrimp in ponds with formalin at 25ppm was reported by Johnson et al. (1973). A lower concentration of formalin (15 ppm), potassium permanganate at 2 and 4 ppm, copper sulfate at 1 ppm, and malachite green at 1 ppm were not effective in other experiments in killing or removing *Zoothamnium* colonies from the shrimp's gills.

A loricate peritrich, probably a *Lagenophrys* sp., has been observed on a general body surface of pond-reared shrimp (*P. setiferus* and *P. vannamei*) in Texas (Johnson, 1974a). At Galveston a similar *Lagenophrys* sp. was observed on the gills of white and brown shrimp. When present on the gills, *Lagenophrys* sp. differs from *Zoothamnium* sp. by evoking a strong cellular inflammatory response. Individual trophonts of *Lagenophrys* sp. typically attach near the tips of the gill lamellae (Fig. 14). While no portion of the lorica appears to penetrate or damage either the cuticle or the underlying hypodermis of the lamellus, the site of attachment becomes heavily inflamed and congested with hemocytes (Fig. 15). Often the hemocyte accumulations become melanized. A similar process of inflammation by hemocytes was noted in the processes of wound repair and foreign body elimi-

nation in the white shrimp (Fontaine and Lightner, 1973; 1974). Shrimp having heavy infestations of *Lagenophrys* sp. on the gills display a "black gill" condition. In such animals, numerous gill lamellae and often large portions of a whole gill process are heavily congested with hemocytes, melanized, and are non-functional. Hence, the respiratory capacity is reduced, and in severely effected animals, death due to suffocation may result if tissue oxygen demands increase (e. g., following handling stress or immediately prior to molting) or if dissolved oxygen levels decrease.

Filamentous Gill Disease.—*Leucothrix mucor* and *Leucothrix*-like filamentous bacocria have been reported from numerous crustaceans. The presence of *Leucothrix mucor* has been demonstrated on the eggs of the rock crab (*Cancer irroratus*), on the setae of the pleopods of the grass shrimp (*Palaemonetes pugio*) and the green crab (*Carcinus maenas*) (Johnson et al., 1971). *Leucothrix*-like filaments have been reported on the surface of developing prawn (*Palaemon serratus*) eggs and on the setae of the pleopods (Anderson and Conroy, 1968). Johnson (1974a) reported a *Leucothrix*-like filamentous bacterium on the general body surface and on the gills of three penaeid species (*P. stylirostris*, *P. setiferus*, and *P. vannamei*) from rearing ponds in Texas. Occasional heavy infestations of this bacterium were noted on the gills.

Barkate et al. (in press) reported mortalities in postlarval penaeid shrimp due to a large filamentous



Figure 14. A *Lagenophrys* sp. attached to the cuticle near the distal end of a gill lamella of *P. aztecus*. No stain. $\times 640$.



Figure 15. Histological section of the gills from *P. aztecus* showing a strong cellular inflammatory response to the two trophonts of *Lagenophrys* sp. shown. Hematoxylin and eosin. $\times 400$.

bacterium. During early stages of development, postlarval shrimp became entangled in filaments of the bacterium, and this entanglement resulted in stress to the shrimp. Direct attachment of the filaments to the carapace region of the postlarval shrimp was also observed. Heavy mortalities (30 to 100%) were experienced, usually suddenly and without warning except for a foul sewage-like odor to the tank. This filamentous bacterium apparently grew on waste materials on the bottom of tanks and was visible as white, cottony mats on the surface of the sediment. The organism was successfully isolated and cultured, but failed to produce filaments on culture media. Reversion to the filamentous state was reportedly not accomplished unless the cultures were inoculated back into the shrimp environment (Barkate et al., in press). In culture Barkate's isolate produced spore-forming rods that stained Gram-positive to Gram-variable. The isolate was tentatively identified as *Bacillus cereus* var. *mycoides*. Infection of healthy postlarval brown and white shrimp was accomplished by addition of the isolate to beakers containing 800 ml of sterilized seawater and 15 shrimp. All of the shrimp which were exposed to the filamentous bacterium died within 48 hours, whereas the control shrimp survived for the duration of the experiment (7 days). The bacterium covered much of the surface of the shrimp at death (Barkate,

et al., in press).

A similar filamentous organism was observed on the gills of juvenile penaeid shrimp in rearing tanks in Florida, and this organism was reportedly isolated on solid media. However, attempts to infect healthy shrimp using "infested tank sediment" rather than the isolate from the gills were not successful (Barkate et al., in press).

Sporadic but serious epizootics due to a *Leucothrix*-like filamentous organism have occurred in tank- and raceway-reared *P. californiensis* at the experimental shrimp farm in Puerto Peñasco, Mexico. Periodic sampling of affected populations of shrimp from the tanks and raceways at Peñasco revealed that the filamentous organism is typically present on the pleopods and gills. When the filamentous organism became so abundant on the gills that respiration was blocked, mortality occurred (Fig. 16).

The filamentous organism at the Puerto Peñasco shrimp farm was initially thought to be either a species of the Oscillatoriaceae group of the blue-green algae or a filamentous bacterium. The latter is believed to be the case, particularly because of the close morphological similarities of this organism to Barkate's isolate (Barkate et al., in press), *Thiothrix marina* (Harold and Stanier, 1955), and *Leucothrix mucor* (Johnson et al., 1971; Bland and Brock, 1973).

The possibility that the filamentous organism in

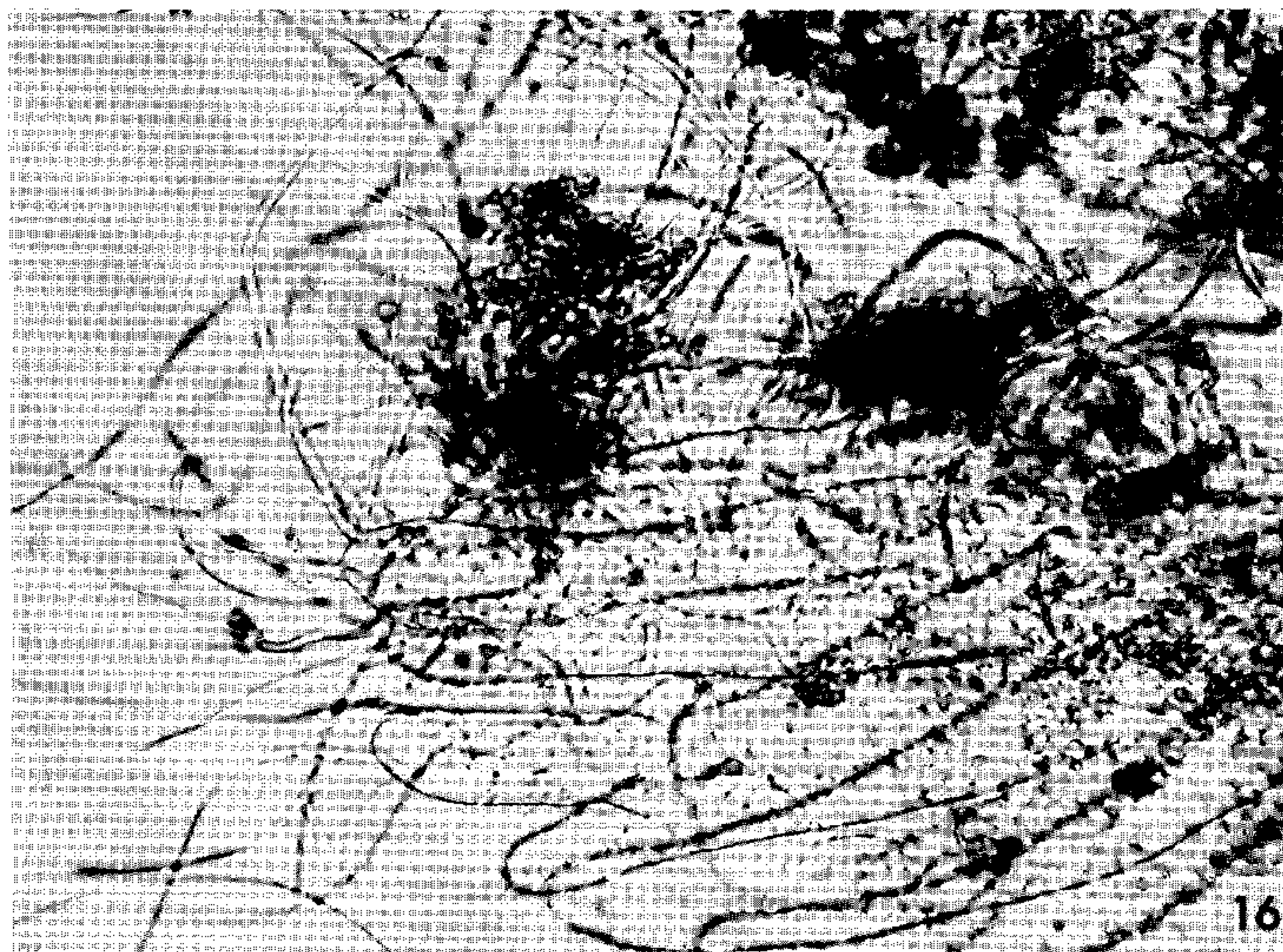


Figure 16. Wet mount preparation of the gills of a brown shrimp (*P. aztecus*) heavily infested with a *Leucothrix*-like filamentous bacterium. Dark areas are debris. No stain. $\times 400$.

Puerto Peñasco may be a blue-green alga was considered because Shelton (1974) described a blue-green alga on the chemoreceptor setae of the North Atlantic brown shrimp (*Crangon crangon*). This organism was similar in morphology to the filamentous organism on the California brown shrimp (*P. californiensis*) in Puerto Peñasco. Shelton (1974) did not culture or attempt to classify the alga from *C. crangon*, beyond placing it in the Oscillatoriaceae. However, a blue-green alga has not been obtained in culture from shrimp having filamentous gill disease.

Histological studies performed on California brown shrimp (*P. californiensis*) from the Puerto Peñasco facility and on brown and white shrimp from Galveston, Texas, revealed that the filamentous organism was strictly external. The organism was attached to the cuticular covering of the gill lamellae, pleopods, or other appendages, and its presence did not result in demonstrable histological damage to underlying tissues (Fig. 17). The filaments themselves appeared to be segmented and had the general appearance of a string of tubular beads (Fig. 18), particularly in histological preparations. The filament segments were anucleate. Trapped in the "mat" formed by the filaments were abundant amounts of detritus, some filamentous green algae, and often numerous diatoms. By itself the filamentous organism is not responsible for discoloration of the gills, but accumulation of algae and debris trapped by the filaments results in

discoloration of the gills that ranges from pale brown to black or green if sufficient algae is trapped by the filaments.

The conditions responsible for the presence of the filamentous organism on the shrimp's gills have not been determined, although low dissolved oxygen levels seem to favor development of the disease, as well as contributing to mortality once the disease has become established. Mortality of shrimp having heavy infestations of the filamentous organism on the gills usually occurs during or immediately following molting. Most of the daily mortalities occurring in epizootics of filamentous gill disease at Puerto Peñasco were "soft shelled". Many premolt animals that showed heavy gill infestations did not survive the next molt if left untreated, despite apparently adequate, but less than saturated dissolved oxygen levels in the water. Animals that did survive the molt shed the filaments with the cast exoskeleton and remained free of the filaments for at least a few days.

Attempts to culture the organism of filamentous gill disease have been made using numerous media, some that are intended for culture of blue-green algae, some for fungi, and the remainder for various types of bacteria. Unfortunately, an organism has been cultured that produces filaments when grown on artificial media that are like those seen in filamentous gill disease. However, one particular

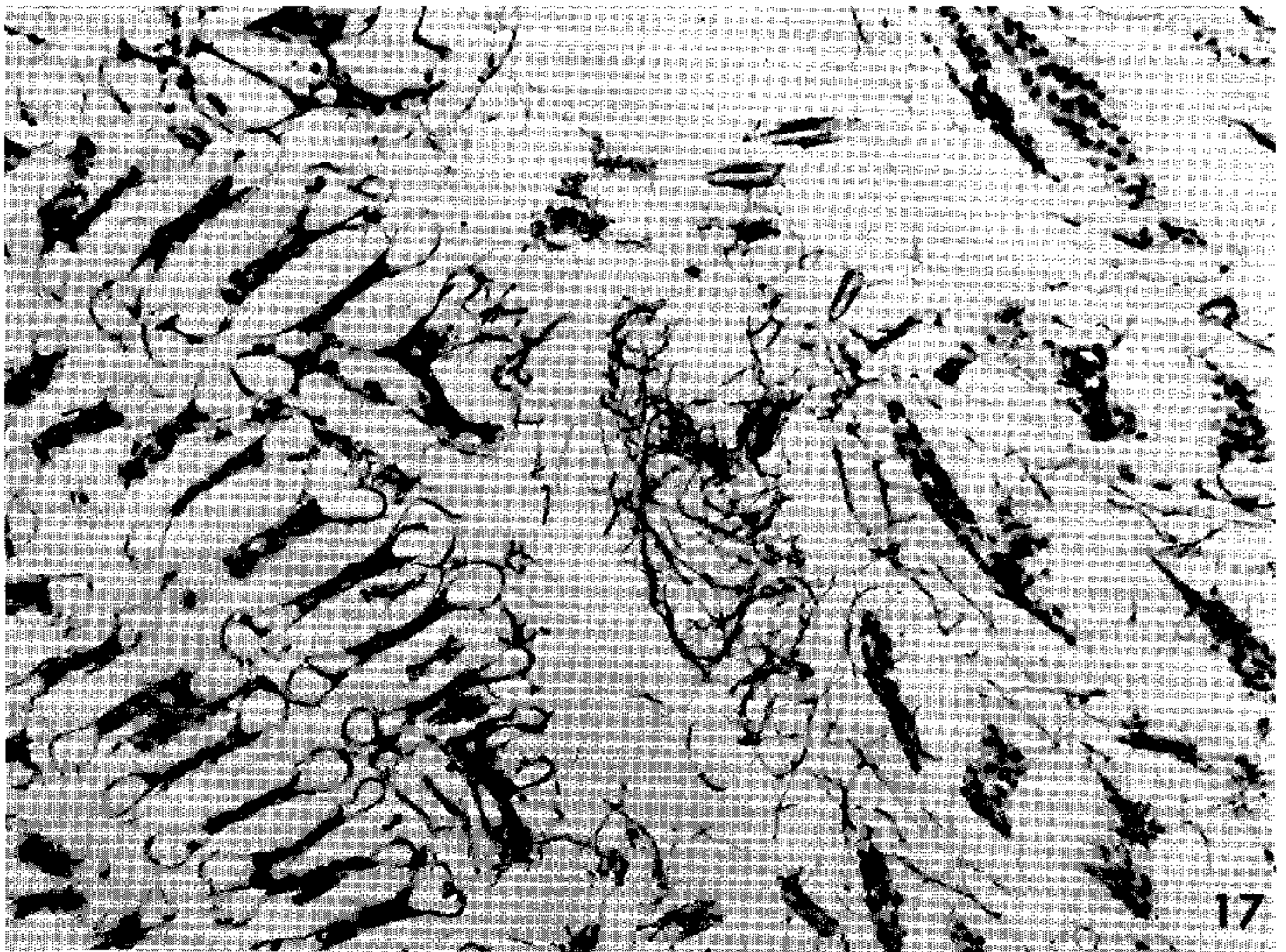


Figure 17. Histological section of the gills of a brown shrimp (*P. aztecus*). Filaments of the *Leucothrix*-like bacterium are abundant on and between the lamellae which show no inflammatory response or histo-pathological change. Hematoxylin and eosin. $\times 180$.

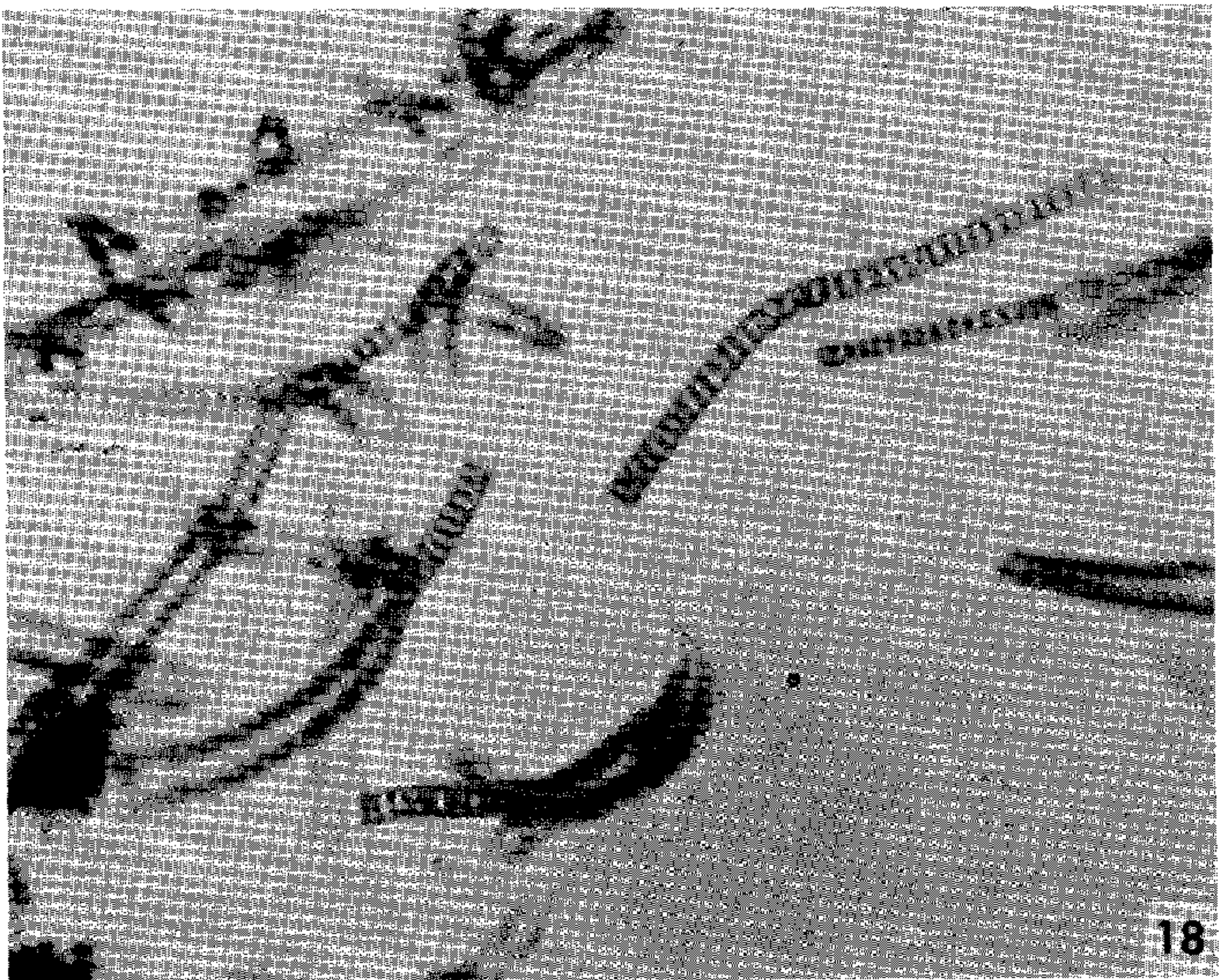


Figure 18. Highly magnified photomicrograph of the *Leucothrix*-like filamentous bacterium from the gills of a California brown shrimp (*P. californiensis*). Note the "string of beads" appearance of the filaments. Formalin fixation, hematoxylin and eosin. $\times 2,000$.

organism has been consistently present in cultures obtained from shrimp having filamentous gill disease in Galveston and in Puerto Peñasco. This organism attracted our attention because it liquified agar, and hence its colonies, when present on certain agar media, lie in pits, while the other organism present do not.

The results of some preliminary experiments indicate that the organism that forms pits on certain agar media such as Harold and Stanier's medium (Harold and Stanier, 1955) is at least associated with filamentous gill disease. When cultured, this organism does not closely resemble the filamentous gill disease organism although it does form long chains and filaments. Additional work is needed to determine whether or not this organism is the causative agent of filamentous gill disease. This organism may belong to the genus *Cytophaga*, to which several pathogens of fish also belong. One disease of hatchery-reared salmonids apparently caused by *Cytophaga* sp. is bacterial gill disease (Bullock, 1972).

Shrimp having filamentous gill disease have been treated successfully with 5 to 10 ppm potassium permanganate in 1-hour static treatments. Occasionally, the use of 10 ppm potassium permanganate has resulted in a chemical toxicity expressed as "burned" gill lamellae which became melanized, necrotic, and were later sloughed. A concentration of 5 ppm potassium permanganate removed or greatly reduced the filaments on the gills, but unfortunately within 5 to 10 days filaments reappeared and mortalities began again. Other chemical treatments including mixtures of malachite green oxalate and formalin at 0.005 to 0.1 ppm and 25 to 75 ppm, respectively, and Hyamine 35000 at 1 to 2 ppm have not been beneficial in 1-hour static treatments. Chemicals such as methylene blue, Roccal, copper sulfate, and Furanace are presently being tested. Also being tested for effectiveness against filamentous gill disease are 24-hour flow-through treatments using 1 to 2 ppm potassium permanganate.

Microsporidian Diseases

The microsporidian parasites of shrimp pose a serious threat to shrimp in open-culture systems such as ponds or the intertidal zones of bays. Aquaculture ventures using tanks or raceways, particularly those operating as closed or semiclosed systems, have not been affected by microsporidian parasites.

At least four species of microsporidia are parasitic to the penaeids of North America and all four have been described from only the Gulf of Mexico and South Atlantic states. *Nosema nelsoni* commonly infects brown and white shrimp (Overstreet, 1973) and the pink shrimp in Florida (Hutton et al., 1959).

Because *N. nelsoni* occurs more commonly than other microsporidia in wild penaeids in the Gulf of Mexico, it may become a more serious problem in commercial shrimp culture operations than will other species of the microsporidia. Shrimp infected with *N. nelsoni* are known as "milk" or "cotton shrimp" due to the chalky white appearance and the "cottony" texture of the musculature (Fig. 19). Histologically, the

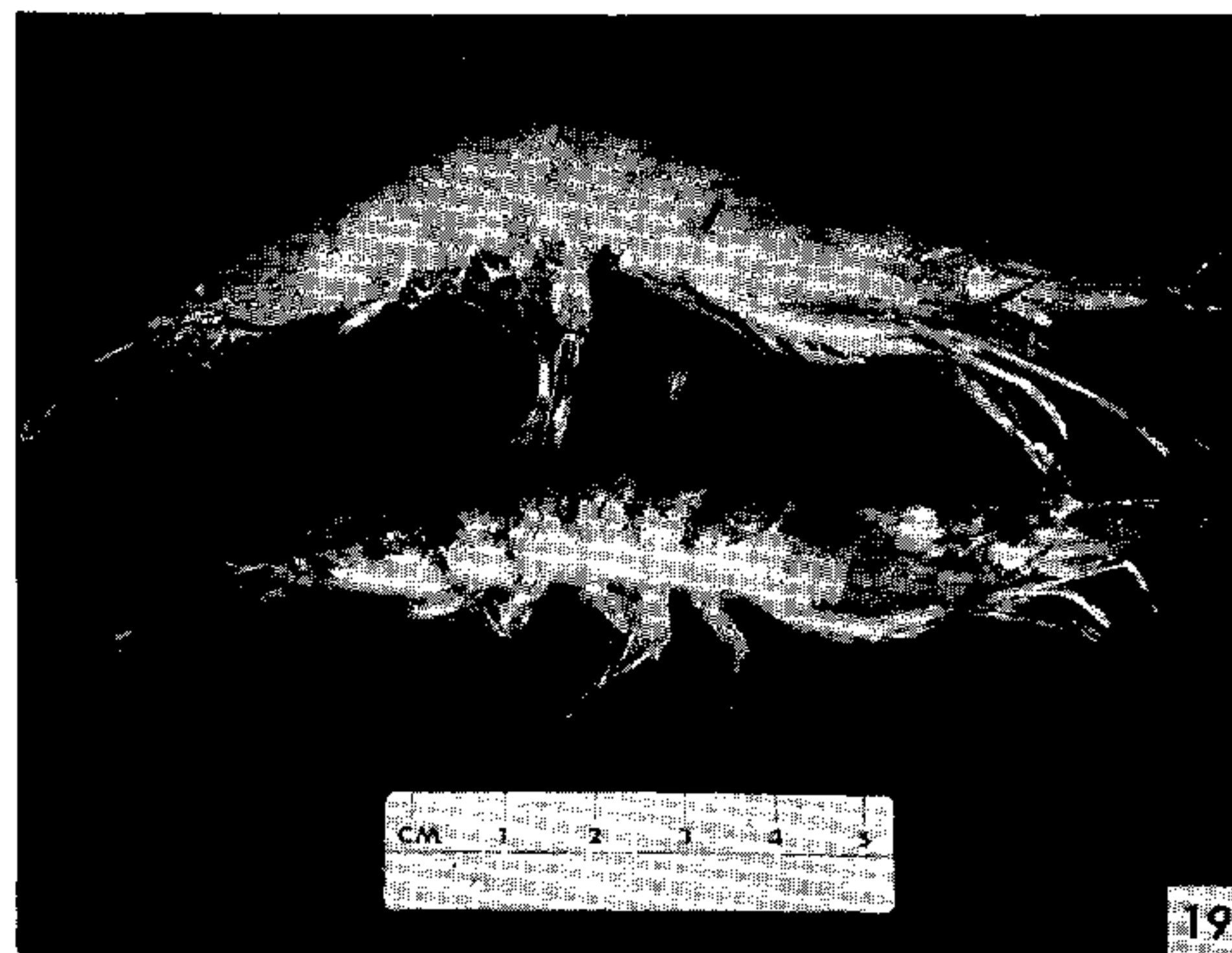


Figure 19. Comparison of a normal shrimp (top) with a "cotton shrimp" (bottom). The cotton shrimp is infected with the microsporidian, *Nosema nelsoni*.

striated muscle fibers become nearly surrounded and eventually are replaced by masses of spores of the parasite (Fig. 20). The fresh spores of *N. nelsoni* are single and average 2.5 μ m in length by 1.5 μ m in width (Overstreet, 1973). Cotton shrimp disease is chronic and gradually debilitates the host. Severely affected animals are common in infected populations of shrimp, but these individuals are more apt to be taken by predators than normal shrimp and are much less resistant to stress. Such shrimp typically do not survive handling, while uninfected shrimp from the same population show good survival. From a commercial viewpoint, cotton shrimp are not a desirable product.

An incidence of 16% cotton shrimp was reported by Elam (personal communication, Texas Parks and Wildlife Department, Palacios, Texas) in 120–130 mm (total length pond-reared brown shrimp in June of 1972. Marifarms in Panama City, Florida, experienced an incidence of about 15% cotton shrimp at harvest in 1971 in white shrimp reared in a net enclosed bay (J. Ikeguchi, personal communication, Marifarms, Panama City, Florida). Since 1971, the incidence of cotton shrimp at Marifarms has been less than 1%.

Another microsporidian, *Pleistophora* sp., causes a similar form of cotton shrimp disease that is grossly

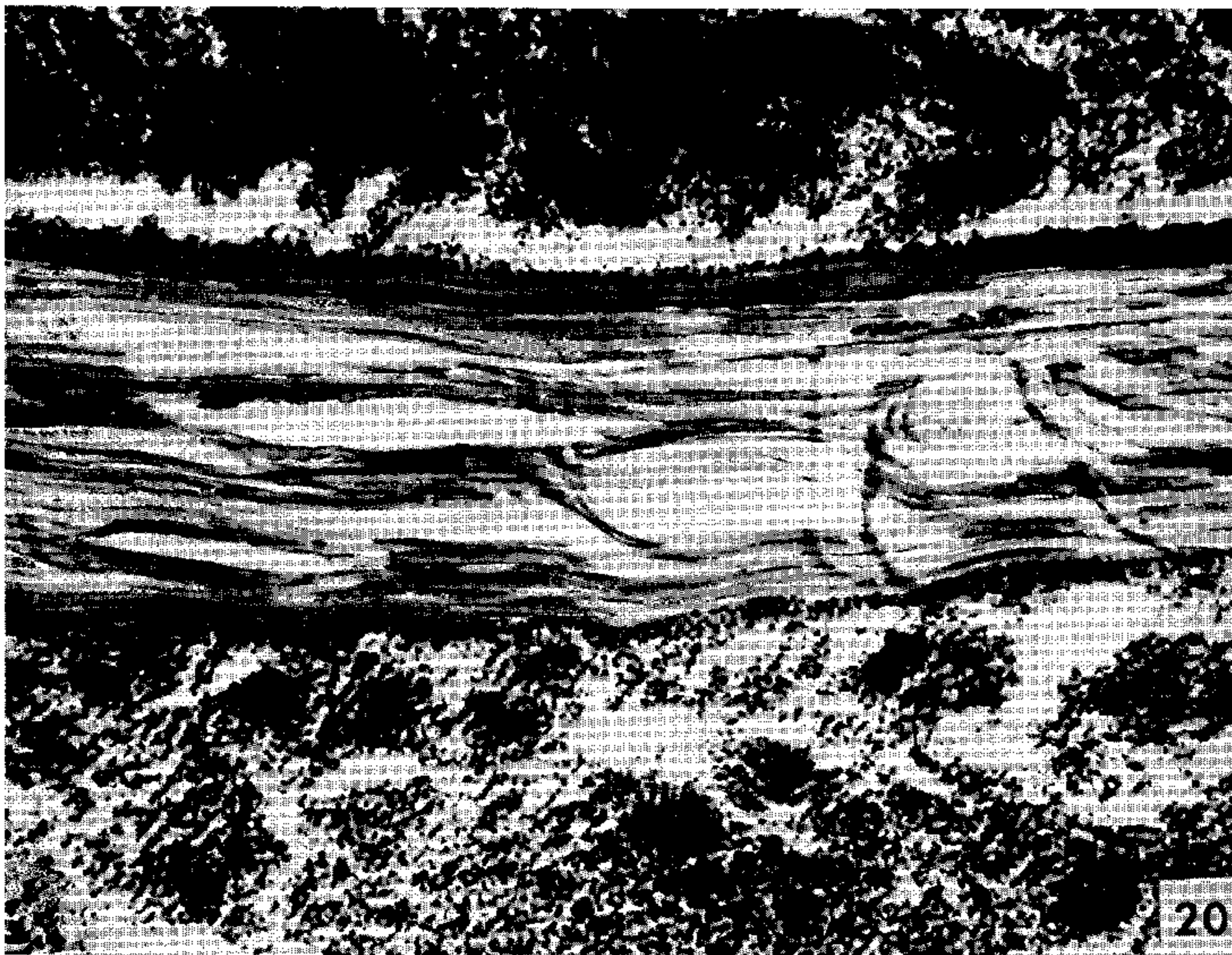


Figure 20. Histological section of tail muscle from a shrimp infected with *Nosema nelsoni*. Giemsa's stain. $\times 900$ (approximate).

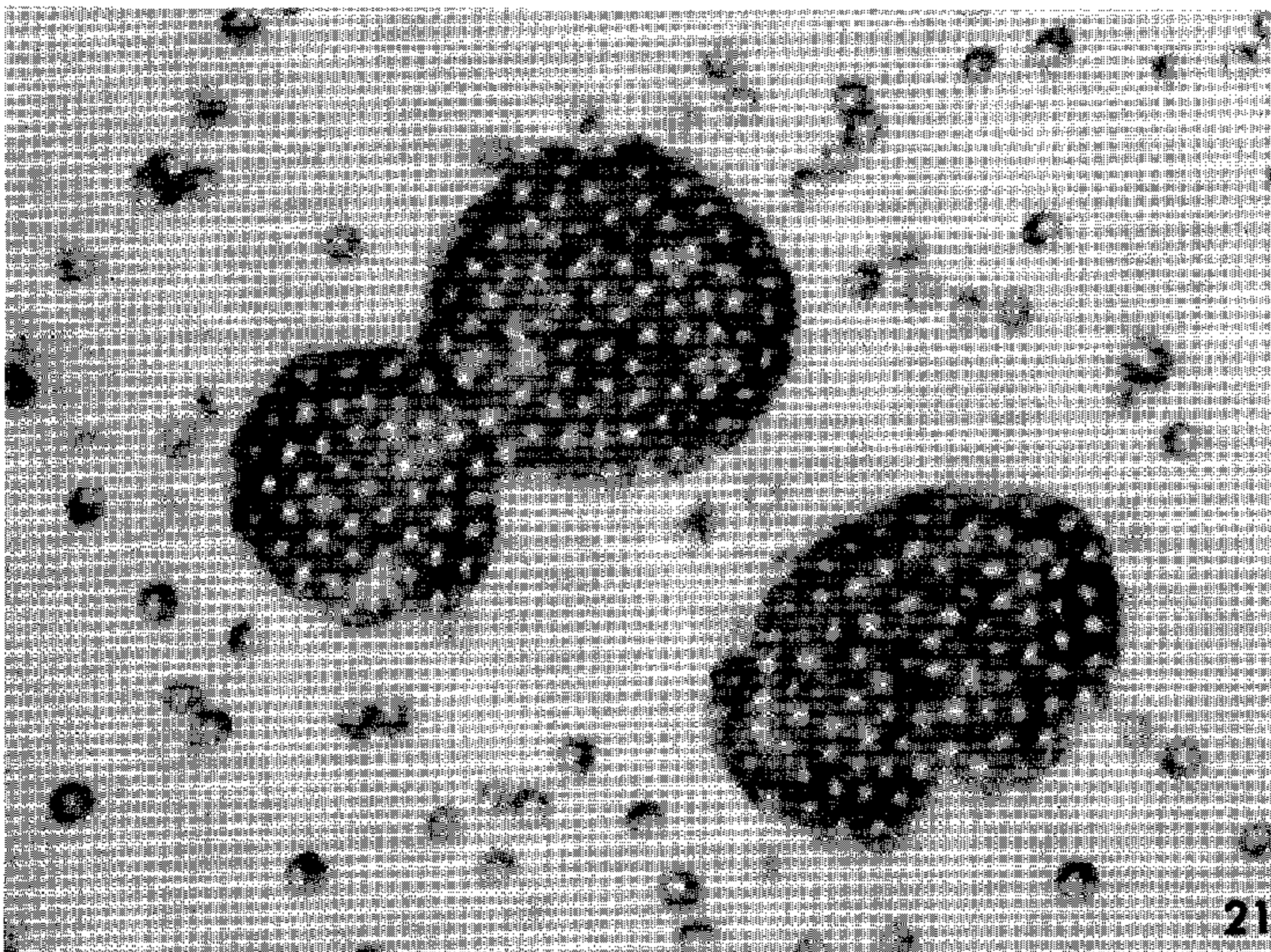


Figure 21. Impression smear of *Pleistophora* sp. from the brown shrimp (*P. aztecus*). Giemsa's stain. $\times 1300$ (approximate).

indistinguishable from infections caused by *N. nelsoni* (Baxter et al., 1970; Overstreet, 1973). The disease has been reported in the brown and white shrimp from Texas (Baxter et al., 1970). Spores of *Pleistophora* sp. develop in sporonts or cysts that are 10 to 55 μm in diameter and contain 14 to hundreds of spores (Fig. 21). Individual fresh spores are slightly pyriform, 2.3 to 3.0 μm long by 1.7 to 2.5 μm wide with a uniform capsule 0.5 μm wide and a polar filament 53–125 μm long by 0.3 μm wide (Overstreet, 1973). This parasite usually replaces the striated muscle tissue, but is also found occasionally in the cardiac muscle, hepatopancreas, gills, and stomach wall muscle (Baxter, et al., 1970).

Pleistophora sp. is not nearly so common in wild shrimp populations as in *N. nelsoni*. It was present in 6.4% of the cotton shrimp sampled from Galveston Bay in 1969 and 1970, while *N. nelsoni* was present in the remainder of the cotton shrimp (Baxter, personal communication, NMFS, Galveston, Texas). *Pleistophora* sp. has not been observed in cultured shrimp on the Gulf of Mexico.

Two additional species of the microsporidia are likely to occur in the culture of penaeid shrimp. These species are *Thelohania duorara* and *T. penaei*.

T. duorara has been reported from pink shrimp and from the Caribbean brown shrimp, *P. brasiliensis* (Iversen and Manning, 1959; Iversen and Van Meter, 1964; Overstreet 1973). Kruse (1959) reported what

is probably the same species in pink, brown, and white shrimp. Affected shrimp have a "cotton shrimp" appearance that is like that caused by *Nosema nelsoni* or *Pleistophora* sp. infections. Spores of *T. duorara* usually lie between muscle fibers, but may completely replace that tissue (Fig. 22). The heart, gonads, and nerve tissues may also be infected. When fresh, the pyriform-shaped spores (Fig. 23) range in size from 4.7 to 6.8 μm long by 3.0 to 4.2 μm wide (average 6.0 by 3.7 μm) with a uniformly wide polar filament 97 to 142 μm long, and eight such spores are in sporonts 8.5 to 13.6 μm in diameter (Overstreet, 1973).

T. penaei infections usually are located along the dorsal midline of the white shrimp. The parasite infects smooth muscles of the blood vessels, foregut, and particularly the germinal tissue of the gonads (Overstreet, 1973). In advanced infections, few if any viable sperm or ova are present in the gonads. The potential effect of this disease is obvious if it were to occur in breeding stock.

The distinctly pyriform spores of *T. penaei* are found in groups of eight in the sporont. There are both microspores which measure 2.5 to 4.7 μm long by 2.0 to 3.5 μm wide, averaging 4.0 by 2.3 μm , and megaspores which are 5.5 to 8.2 μm by 3.5 to 4.2 μm . The polar filaments protrude 65 to 87 μm , averaging 74 μm , with a uniformly thick proximal portion and a thin distal portion following a transitional tapering zone. Sporonts are 7 to 12 μm wide, averaging 9.3 μm

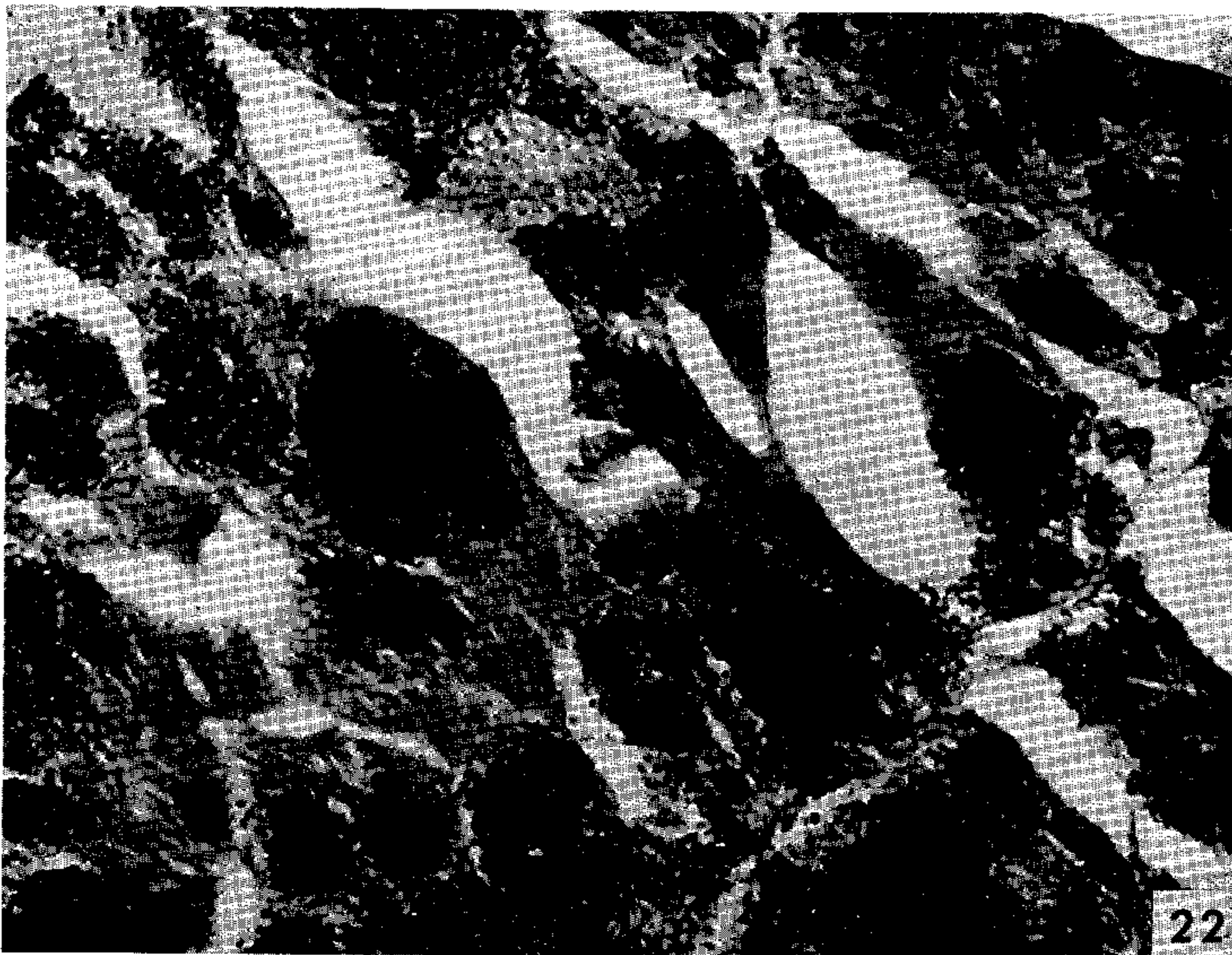


Figure 22. Photomicrograph of *Thelohania duorara* in the tail muscle of a brown shrimp, (*P. aztecus*). Giemsa's stain. $\times 640$.

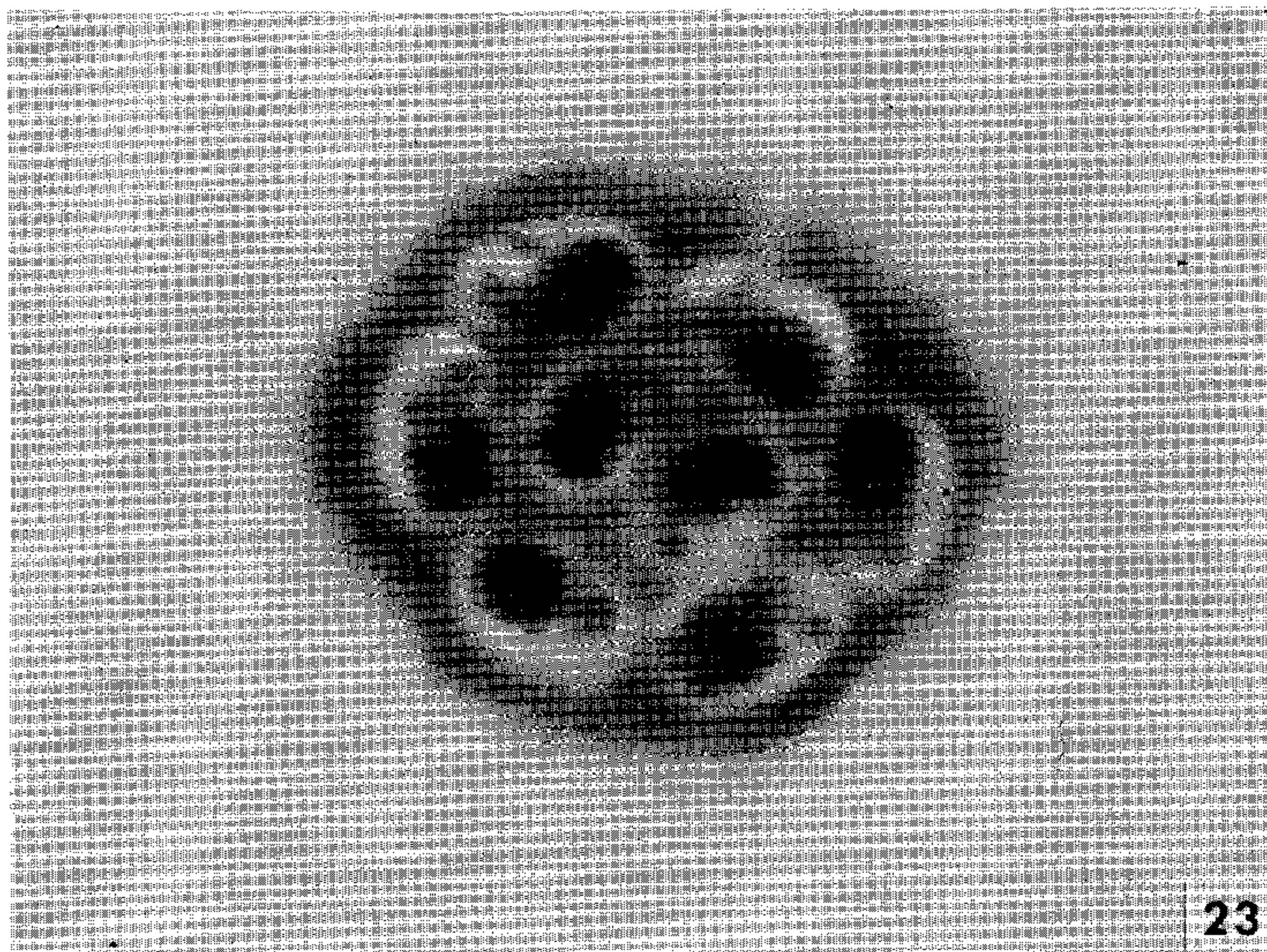


Figure 23. Photomicrograph of an impression smear of the gonad of a male white shrimp (*P. setiferus*) infected with *Thelohania penaei*. Giemsa's stain. $\times 5,500$ (approximate).

(Overstreet, 1973).

Shrimp can be infected with more than one species of microsporidian. Individual white shrimp (*P. setiferus*) infected with *T. penaei*, *N. nelsoni*, and *Pleistophora* sp. were reported from Mississippi (Overstreet, 1973). At Galveston, brown shrimp from Galveston Bay have been found to possess dual infections of *N. nelsoni* and *T. duorura* in the tail musculature.

There are no known treatments for shrimp infected with any of the microsporidia. Furthermore, the means of transmission from host to host and even the method by which shrimp become infected remain to be discovered (Roth and Iversen, 1971).

SUMMARY

At least five major diseases of cultured penaeid shrimp are recognized as potential obstacles to successful commercial culture of penaeid shrimp. These diseases are:

1. A mycosis of larval penaeid shrimp caused by a *Lagenidium* sp. This disease has been recognized in larval white (*Penaeus setiferus*) and brown (*P. aztecus*) shrimp in Texas and in Florida. The protozoal larval stages are the most severely affected by the fungus with losses of nearly 100% reported. No methods of chemical or drug therapy have been developed for treatment of the disease.

2. A mycotic infection of juvenile penaeid shrimp with *Fusarium* spp. have been reported from *P. japonicus* in Japan, *P. duorarum* and possibly *P. aztecus* in Texas, and in *P. californiensis* in Puerto Peñasco, Sonora, Mexico. High mortalities accompanied the disease in *P. japonicus* and *P. californiensis* and affected shrimp frequently possessed "black gill". As is the case with the mycosis caused by *Lagenidium* sp., no methods of therapy have been reported in treating shrimp infected with *Fusarium* spp., but control of the disease was accomplished at Puerto Peñasco by elimination of the source of the fungus and by destruction of infected stock.

3. Bacterial infections caused by *Vibrio* spp. and *Beneckea* spp., *V. parahaemolyticus*, *V. alginolyticus*, and *V. anguillarum* have been implicated as the cause of severe bacteremic epizootics in cultured penaeid shrimp in Texas. Losses due to *Vibrio* infections have reportedly reached nearly 100%. Certain antibiotics have possible beneficial therapeutic effects in treatment of *Vibrio* infections when added directly to the ration or to the water.

"Shell disease" is a complex of diseases expressed as cuticular lesions that typically become melanized. Chitinoclastic bacteria belonging to the genera *Beneckea*, *Vibrio*, and *Pseudomonas* have been isolated from shrimp having shell disease. As with bacteremic infections due to *Vibrio* spp., bacterial "shell disease"

has been treated successfully in preliminary experiments in Puerto Peñasco using antibiotic therapy administered with the ration. Addition of mixtures of malachite green oxalate and formalin to the water in static 1-hour treatments has also shown a beneficial effect in preliminary tests.

4. Gill disease. Gill disease in penaeid shrimp is a complex of several diseases, all of which may result in death of affected shrimp by destruction of the gills or by suffocation due to mechanical blockage of gas exchange across the surface of the gill lamellae. Organisms demonstrated to cause gill disease in penaeids include *Fusarium* spp., *Zoothamnium* sp., *Lagenophrys* sp., and a *Leucothrix*-like filamentous bacterium. Frequent and high losses of shrimp have been experienced in Texas, Florida, and Puerto Peñasco, due to members of this complex of gill diseases. Successful therapy using formalin has been reported for pond-reared shrimp having an infestation of the gills by *Zoothamnium* sp. in Texas. The severity of epizootics of "filamentous gill" disease due to a *Leucothrix*-like filamentous bacterium in Puerto Peñasco has been temporarily reduced in raceway and tank-reared shrimp by addition potassium permanganate to the water.

5. Microsporidian disease. At least four species of microsporidia are parasitic to the penaeids of North America. *Nosema nelsoni*, *Pleistophora* sp., *Thelohania duorara*, and *T. penaei* cause a chronic disease in penaeids that is commonly referred to as "cotton shrimp" disease due to the cottony appearance of the tissues infected by the parasite. No effective treatments have been reported for this group of parasites in shrimp, nor has the means of transmission from shrimp to shrimp been discovered.

ACKNOWLEDGMENTS

The valuable assistance of C. T. Fontaine, L. Wible, and I. Sanderson of the National Marine Fisheries Service, Galveston, Texas, is thankfully acknowledged. K. N. Baxter of the same address kindly granted permission for inclusion of the prints used as Figures 19, 20, 21, and 23.

Kenneth Hanks and Leon Armando Perez Alvidrez of the University of Arizona-University of Sonora, Mexico Experimental Shrimp Culture Station at Puerto Peñasco, Sonora, Mexico, are acknowledged for technical assistance. Dr. D. H. Lewis is thankfully acknowledged for his assistance in classifying the bacterial isolates listed in Table 1.

LITERATURE CITED

- ANDERSON, J. I. W., and D. A. CONROY: 1968. The significance of disease in preliminary attempts to raise crustacea in sea water. *Bull. Off. int. Epiz.* 69, 1239-1247.
- BARKATE, JOHN A: 1972. Preliminary studies of some shrimp diseases. *Proc. 3rd Ann. Workshop, World Mariculture Society*, 337-346.
- BARKATE, JOHN A., YOSUKE HIRONO, and HARVEY PERSYN: In Press. Some marine micro-organisms related to shrimp diseases. *Proc. 5th Ann. Workshop, World Mariculture Society, Charleston, South Carolina*, 1974.
- BAXTER, KENNETH N., R. H. RIGDON, and CONSTANCE HANNA: 1970. *Pleistophora* sp. (Microsporidia: Nosematidae): A new parasite of shrimp. *J. Invertebr. Pathol.*, 16, 289-291.
- BLAND, C. E: 1974. A survey of fungal diseases of marine organisms with emphasis on current research concerning *Lagenidium callinectes*. *Proc. Gulf Coast Regional Symposium on Diseases of Aquatic Animals, Baton Rouge, Louisiana*. LSU-SG-74-05, 47-51.
- BLAND, J. A., and T. D. BROCK: 1973. The marine bacterium *Leucothrix mucor* as an algal epiphyte. *Marine Biology* 23, 283-292.
- BULLOCK, G. L: 1972. Studies on selected Myxobacteria pathogenic for fishes and on bacterial gill disease in hatchery-reared salmonids. *Bureau of Sport Fisheries and Wildlife Technical Paper*, No. 60.
- CHAN, EVA S., and ADDISON W. LAWRENCE: In Press. Effect of antibiotics on the respiration of brown shrimp larvae to postlarvae and bacterial populations associated with shrimp. *Proc. 5th Ann. Workshop, World Mariculture Society, Charleston, South Carolina*, 1974.
- COOK, H. L: 1971. Fungi parasitic on shrimp. *FAO Aquaculture Bull.*, 3, 13.
- COOK, David W., and SANDRA R. LOFTON: 1973. Chitino-clastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab (*Callinectes sapidus*). *J. of Wildlife Diseases*, 19, 154-159.
- CORLISS, JANE, Z. P. ZEIN-ELDIN, and D. V. LIGHTNER: In Press. Effect of terramycin on growth and survival and its use in disease control in *Penaeus aztecus*. *Proc. 5th Ann. World Mariculture Society, Charleston, South Carolina*, 1974.
- COUCH, J. H: 1942. A new fungus on crab eggs. *J. Elisha Mitchell Sci. Soc.*, 58, 158-162.
- DELVES-BROUGHTON, J: 1974. Preliminary investigations into the suitability of a new chemotherapeutic, Furanace, for the treatment of infectious prawn diseases. *Aquaculture*, 3, 175-185.
- EDDY, B. P: 1960. Cephalotrichous, fermentative Gram negative bacteria: the genus *Aeromonas*. *J. Appl. Bact.*, 23: 216-249.
- EDDY, B. P., and K. P. CARPENTER: 1964. Further studies on *Aeromonas*. II. Taxonomy of *Aeromonas* and C27 strains. *J. Appl. Bact.*, 27, 96-109.
- EGUSA, S., and T. UEDA: 1972. A *Fusarium* sp. associated with black gill disease of the Kuruma prawn, *Penaeus japonicus* Bate. *Bulletin of the Japanese Society of Scientific Fisheries*, 38, 1253-1260.

- FONTAINE, C. T. and D. V. LIGHTNER: 1973. Observations on the process of wound repair in penaeid shrimp. *J. Invertebr. Pathol.*, 22, 23-33.
- FONTAINE, C. T. and D. V. LIGHTNER: 1974. Observations on the phagocytosis and elimination of carmine particles injected into the abdominal musculature of the white shrimp, *Penaeus setiferus*. *J. Invertebr. Pathol.*, 24, 141-148.
- HAROLD, R., and R. Y. STANIER: 1955. The genera *Leucothrix* and *Thiothrix* *Bacteriol. Rev.*, 19, 49-58.
- HOOD, MARY A., and SAMUEL P. MEYERS: In Press. Implications of chitin and chitinase activity in response of crustacea and fishes to variously formuly formulated diets. *Proc. 5th Ann. Workshop, World Mariculture Soc., Charleston, S. C., 1974.*
- HUTTON, ROBERT F., FRANKLIN SOGANDARES-BERNAL, BONNIE ELDRED, ROBERT M. INGLE, and KENNETH D. WOBDURN: 1959. Investigations on the parasites and diseases of saltwater shrimps (Penaeidae) of sports and commercial importance to Florida. *State of Florida, Board of Conservation Marine Laboratory, Technical Series, No. 26.*
- IVERSEN, E. S., and R. B. MANNING: 1959. A new microsporidian parasite from the pink shrimp (*Penaeus duorarum*). *Trans. Amer. Fish. Soc.*, 88: 130-132.
- IVERSEN, E. S., and N. H. VAN METER: 1964. A record of the microsporidian, *Thelohania duorara*, parasitizing the shrimp *Penaeus brasiliensis*. *Bull. Mar. Sci. Gulf and Caribbean*, 14: 549-553.
- JOHNSON, PAUL W., JOHN MCN. SIEBURTH, AKELLA SASTRY, C. R. ARNOLD, and MAXWELL D. DOTY: 1971. *Leucothrix mucor* infestation of benthic crustanea, fish eggs, and tropical algae. *Limnol. and Oceanogr.*, 16: 962-969.
- JOHNSON, S. K., J. C. PARKER, and HOYT W. HOLCOMB: 1973. Control of *Zoothamnium* sp. on penaeid shrimp, *Proc. 4th Ann. Workshop, World Mariculture Society*, 321-331.
- JOHNSON, S. K.: 1974a. Ectommensals and parasites of shrimp from Texas rearing ponds. *Texas A & M University, Sea Grant Publication*, No. TAMU-56-74-207, 20 pp.
- JOHNSON, S. K.: 1974b. *Fusarium* sp. in laboratory-held pink shrimp. *Fish Disease Diagnostic Laboratory-S1. Texas A & M University.*
- JOHNSON, T. W., JR: 1958. A fungus parasite in ova of the barnacle *Chthamalus fragilis denticulata*. *Biol. Bull.*, 114: 205-214.
- JOHNSON, T. W., JR. and RUPERT R. BONNER, JR: 1960. *Lagenidium callinectes* Couch in barnacle ova. *J. Elisha Mitchell Sci. Soc.*, 76: 147-149.
- KRUSE, DWAYNE NATHANIEL: 1959. Parasites of the commercial shrimps, *Penaeus aztecus* Ives, *P. setiferus* (Linnaeus). *Tulane Studies in Zoology*, 7: 123-144.
- LEWIS, D. H.: 1973a. Response of brown shrimp to infection with *Vibrio* sp. *Proc. 4th Ann. Workshop, World Mariculture Society*, 333-338.
- LEWIS, D. H.: 1973b. Predominant aerobic bacteria of fish and shellfish. *Texas A & M University. Sea Grant Publication*, No. 401. 102 pp.
- LIGHTNER, D. V. and C. T. FONTAINE: 1973. A new fungus disease of the white shrimp *Penaeus setiferus*. *J. Invertebr. Pathol.*, 22: 94-99.
- LIGHTNER, DONALD V: In Press. A mycosis of cultured lobsters (*Homarus americanus*) caused by a *Fusarium* sp. *FAO Aquaculture Bulletin.*
- LIGHTNER, DONALD, and C. T. FONTAINE: 1975. A *Fusarium* sp., apparent cause of a mycosis in the American lobster (*Homarus americanus*). *J. Invertebr. Pathol.*, 25: 239-245.
- LIGHTNER, DONALD V., and DONALD H. LEWIS: 1975. A septicemic bacterial disease syndrome of penaeid shrimp. *In: Diseases of Crustaceans. Marine Fisheries Review*, 37: 25-28.
- NEAL, RICHARD A.: 1973a. Progress toward farming shrimp in the United States. *Marine Fisheries Review*, 35: 67-70.
- NEAL, R. A.: 1973b. Alternatives in aquacultural development: consideration of extensive versus intensive methods. *J. Fish. Res. Board Canada*, 30: 2218-2222.
- NICKELSON, R. and C. VANDERZANT: 1971. *Vibrio parahaemolyticus*-a review. *J. Milk Food Technol.*, 34: 447-452.
- OVERSTREET, ROBIN M: 1973. Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture*, 2, 105-140.
- ROGERS-TALBERT, R.: 1948. The fungus *Lagenidium callinectes* Couch (1942) on the eggs of the blue crab in Chesapeake Bay. *Biol. Bull.*, 95: 214-228.
- ROSEN, BARUCH: 1970. Shell disease of aquatic crustaceans. *In: A Symposium on Diseases of Fishes*, S. F. Snieszko, editor. *Amer. Fish. Soc. Sp. Publ.*, No. 5, 409-415.
- ROTH, J. N., and E. S. Iversen: 1971. Attempts to transmit experimentally the microsporidian *Thelohania duorara*, parasitizing the pink shrimp, *Penaeus duorarum*. *Trans. Amer. Fish. Soc.*, 100: 369-370.
- SCHUBERT, R. H. W.: 1962. Zur technik der differenzierung von vibrionen und pseudomonaden mit dem vibriostaticum 0/129, *Zent. Bak. Parasit. Abt.*, 1 Orig. 184: 560-561.
- SCHUBERT, R. H. W.: 1967. The taxonomy and nomenclature of the genus *Aeromonas* Kluyver and van Niel 1936. *Int. J. Syst. Bact.*, 17: 23-27.
- SHELTON, R. G. J.: 1974. Observations on the occurrence of an epizootic, blue-green alga on the chemoreceptor setae of the brown shrimp, *Crangon crangon* (L.). *J. Mar. Biol. Ass. U. K.*, 54: 301-307.
- SINDERMAN, C. J.: 1971. Disease-caused mortalities in mariculture, status and predictions. *Proc. Second Annual Workshop World Mariculture Society*, 69-74.
- SINDERMAN, C. J.: 1974. *Vidrio* (*V. parahaemolyticus*) disease of juvenile and adult shrimps. *In: Handbook of Diagnosis and Control of Diseases in Mariculture. U. S. Dept. of Commerce, N. M. F. S. Middle Atlantic Coastal Fisheries Center. Informal Report*, No. 19.
- SNIESZKO, S. F. and C. C. TAYLOR: 1947. A bacterial disease of the lobster (*Homarus americanus*). *Science*, 106: 500.
- STEWART, J. E., and H. RABIN 1970. Gaffkemia, a bacterial disease of lobsters (genus *Homarus*). *In: A symposium on diseases of fishes and shellfishes*, S. F. Snieszko, editor. *Spec. Publ. Ann. Fish. Soc.*, No. 5, 431-439.
- UZMAN, JOSEPH R. and EVAN B. HAYNES: 1968. A mycosis

- of the gills of the pandalid shrimp, *Dichelopandalus leptopus*. *J. Invertebr. Pathol.*, 12: 275-277.
- VANDERZANT, C., R. NICKELSON, and J. C. PARKER: 1970a. Isolation of *Vibrio parahaemolyticus* from Gulf coast shrimp. *J. Milk Food Technol.*, 33: 161-162.
- VANDERZANT, CARL, EVA MROZ, and R. NICKELSON: 1970b. Microbial flora of Gulf of Mexico and pond shrimp. *J. Milk Food Technol.*, 33: 346-350.
- VANDERZANT, C., R. NICKELSON, and P. W. JUDKINS: 1971. Microbial flora of pond-reared brown shrimp (*Penaeus aztecus*). *Appl. Microbiol.*, 21: 916-921.

List of Corrections

<u>Page</u>	<u>Column</u>	<u>Paragraph</u>	<u>Line</u>	<u>Error</u>	<u>Correction</u>
75	1	1	11	Peñascol	Peñasco
	1	2	5	disease	diseases
78	1	1	2	did no	did not
	1	2	11	one latter	the latter
	2	2	19	shirimp	shrimp
81	2	3	3	air-inflaeted	air-inflated
82	2	3	13	<i>V. Anguil-</i>	<i>V. anguil-</i>
84	1	4	1	is possible the	is possible by the
85	2	1	9	<i>Laucothrix</i>	<i>Leucothrix</i>
89	2	3	5	on organism	no organism
91	1	3	13-14	Hyamine 35000	Hyamine 3500
93	1	2	2	as in <i>N. nelsoni</i>	as is <i>N. nelsoni</i>
95	1	3	6	diasese	disease
	1	5	6	or his	for his

REFERENCES

96	Hood and Meyers Sindermann 1974	formuly Vidrio	(omit) Vibrio
----	------------------------------------	-------------------	------------------

FIGURES & TABLES

80	Figure 6 (last line)	cncapsulations	encapsulations
83	Table 1, footnote 1	(<i>Penaeus aztecus</i>)	(<i>Penaeus aztecus</i>)